The Journal of Laboratory and Clinical Medicine

VOL. XVIII ST. LOUIS, MO., APRIL, 1933 NO. 7

CLINICAL AND EXPERIMENTAL

THE HEMORRHAGIC TENDENCY IN JAUNDICE: A STUDY OF THE BLOOD FIBRIN, SEDIMENTATION RATE, COAGULATION TIME, AND OTHER BLOOD FACTORS*

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THE hemorrhagic tendency of the jaundiced patient sometimes causes much concern. By the careful preoperative preparation outlined by Walters,[37, 38] the operative mortality in jaundice has been reduced. However, there are patients who tend to bleed in spite of these preventive measures. Little is known regarding this hemorrhagic tendency. In an effort to add to knowledge of this subject, various aspects of the blood of jaundiced patients were studied as well as of other patients who could be considered as controls. These studies included blood fibrin, sedimentation rate of erythrocytes, coagulation time of the blood, and bilirubin, protein, and viscosity of the serum. Only cases of jaundice of obstructive or intrahepatic type have been considered in this work.

METHODS AND NORMAL VALUES

In our study, the method of Foster and Whipple was used for determinations of blood fibrin, except that "oxalated tubes" were used instead of sodium oxalate solution. These tubes were prepared in the following manner: Of a 3.2 per cent solution of sodium oxalate, 1 c.c. was added to each of the 15 c.c. graduated centrifuge tubes. These were then placed in a sand bath, and in the process of drying, the sodium oxalate solution was spattered on the sides of the tubes. Another vari-

*Abridgment of thesis submitted by Dr. Burke to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine. Work done while a Fellow in Medicine, The Mayo Foundation.

Received for publication, May 26, 1932.

ation from the method of Foster and Whipple concerned precipitation and collection of the fibrin. When the fibrin was completely coagulated it was freed from the walls of the tube by a glass rod. The tube was then centrifugalized at a rapid rate, until the fibrin was closely packed in the bottom of the tube, in a button-like formation. In the remaining details their method was closely followed.* We found that in men the concentration of fibrin ranges normally between 170 and 340 mg., and in women, between 280 and 360 mg. in each 100 c.c. of blood. These values are somewhat higher than those noted by Foster.

In our work on sedimentation of erythrocytes we followed the method of Plass and Rourke, with the exception of one aspect of it, because of its simplicity and because heparin is used for the anticoagulant. Instead of using collection tubes prepared as described, we added to each collection tube, immediately before it was used, 0.1 c.c. of a 3 per cent solution of heparin (containing 3 mg. of anticoagulant). This was done because the heparin, dried in the tube as suggested, did not uniformly prevent coagulation. The normal range of sedimentation rate for women, determined by us, was 3 to 29 mm. per hour, or 5 to 58 per cent. For men it was 2 to 18 mm. per hour, or 5 to 34 per cent. These figures are somewhat higher than those of Plass and Rourke.

The method of Lee and White was used in our study for determination of the coagulation time of the blood. Lee and White gave as the average normal coagulation time six and a half minutes, with five to eight minutes as the limits of normal. Our results were similar for normal persons. We have arbitrarily refused to consider the coagulation time sufficiently prolonged to be of clinical significance if it is less than twelve minutes.

Van den Bergh concluded that two types of reaction were seen in determinations of serum bilirubin by his method: one was obtained immediately after addition of the diazo reagent, a "direct reaction," and the other after addition of the diazo reagent and alcohol, an "indirect reaction." Two other types of reaction are described which are probably not of clinical significance, the "delayed reaction" and the "biphasic reaction." The delayed reaction commences only after one to fifteen minutes or longer, and consists in the development of a reddish coloration, which gradually deepens and becomes more violet. In the biphasic reaction a slight reddish color appears immediately (ten to thirty seconds) which, after a minute or a much longer time, deepens gradually and becomes more violet. The delayed reaction is reported as "indirect" and the biphasic as "direct." The Thannhauser and Anderson modification of the indirect van den Bergh reaction is used in the laboratories of The Mayo Clinic for quantitative determination of the amount of bilirubin in the blood serum. The results are reported in milligrams of bilirubin for each 100 c.c. of serum.

For determination of proteins of the serum, the refractometric method of Reiss, as modified by Neuhausen and Rioch, was used. The viscosimeter of Hess was employed for determining the viscosity of the serum. Bircher gave 1.7 to 2.0 as the normal range for viscosity of the serum. Our values for normal persons fell within these limits.

*A few of our determinations of fibrin were made by the method of Schultz, Nicholes, and Schaefer. The difference between the results of the two methods is of little consequence.

CORRELATION OF FACTORS IN JAUNDICE IN REGARD TO HEMORRHAGIC TENDENCY

Snell, Greene and Rowntree studied the blood fibrin of experimental animals in which obstructive jaundice was produced. They found a sharp rise postoperative in all animals, with gradual return to normal for several days. Thereafter the values approached the upper limits of normal as a rule. Occasionally high values were observed, but in no instance was there a definite decrease in fibrin. They observed no direct relationship between coagulation time and fibrin content of the blood. In each of four cases of obstructive jaundice due to carcinoma, Gram found high values for blood fibrin. Of four cases of catarrhal jaundice, in only one was there an increase in fibrin. In another series, however, Gram found the blood fibrin increased in four cases of catarrhal jaundice. Mann, Bollman, and Markowitz attributed the delayed coagulation time in dogs following hepatectomy to deficiency in fibrinogen of the blood. However, they found no simple relationship between the content of fibrinogen of the blood and the coagulation time. According to Starlinger, the fibrinogen and globulin of the blood in jaundice seem to be increased, except in grave icterus, in which the fibrinogen is greatly diminished.

The sedimentation rate is influenced by many factors. Fåhraeus noticed pronounced parallelism between the amount of fibrinogen in the blood, or the serum globulin, and the sinking velocity of the corpuscles. Hunt[11, 12] also found a definite relationship between the fibrin content of the blood and the rate of sedimentation.

A similar relationship has been noted by many other workers, including Greisheimer,[6, 7, 8] Ryan and Johnson, Gram, and Rourke and Plass. On the other hand, Pinner, Knowlton and Kelly have expressed the belief that there is no definite relationship between the blood fibrin and the sedimentation rate of the erythrocytes. The sedimentation is affected, also, by the number of erythrocytes, the cell volume, and the concentration of hemoglobin. Hunt[11, 12] could establish no definite ratio between the erythrocyte count and the sedimentation rate; nevertheless, he expressed the belief that variation of the cell count to any great extent, either above or below normal limits, affected the sedimentation test. By decreasing the cell volume he found that normal sedimentation became more rapid, and by reversing this process, that normal sedimentation became slower. His opinion was that the sedimentation rate was influenced by anemia to such an extent that it could not be relied on in such cases. Rubin and Smith, using hirudinized blood, noted close relationship between the erythrocyte count, the sedimentation rate, and the cell volume as determined by the hematocrit. The lower the cell volume the greater was the rapidity of sedimentation. Similar relationships have been found by other workers, including Hubbard and Geiger, Gram, and Greisheimer, Ryan and Johnson. Various methods have been described for the correction of the sedimentation rate made inaccurate by variations in number of erythrocytes, concentration of hemoglobin, and cell volume. The methods of Plass and Rourke, and of Gram, deserve mention. Greisheimer discovered no significant difference in the sedimentation rate during the menstrual flow from that in the intervals between periods of flow. Lee and White, in 1913, suggested a relationship between the coagulation time and the settling of the corpuscles as demonstrated by the "buffy coat." Ro-

senthal and Blowstein found the sedimentation rate increased in 80 per cent of the cases of jaundice in their series. They expressed the belief that the sedimentation rate has no relation to the degree or duration of jaundice or of biliary obstruction. They noticed normal sedimentation rates in a number of cases of catarrhal jaundice, and concluded that a normal sedimentation time, if a patient were jaundiced and without polycythemia, is suggestive of catarrhal jaundice. Linton noted correlation between the sedimentation rate and the hemorrhagic tendency in jaundice. In his work he divided postoperative hemorrhage among patients with obstructive jaundice into primary or early hemorrhage occurring immediately after operation, and secondary or delayed hemorrhage becoming manifest a week or ten days after operation. He found that the preoperative sedimentation rate in three of four cases in which there was primary bleeding was extremely rapid. In ten cases in which there was no primary hemorrhage the sedimentation rate was slow in all except one, and in this case fever was present. The sedimentation rate was taken at the time of the hemorrhage in seven cases. The rate in all cases was very rapid. Only one of the patients had fever, which might have accounted for the increased sedimentation rate. Postoperative determinations gave some indication as to the possibility of delayed bleeding. Linton expressed the belief that the sedimentation rate of the blood is a more reliable test of the hemorrhagic tendency in jaundice than determinations of coagulation time and of bleeding time.

The coagulation time has always been studied in consideration of the hemorrhagic tendency of jaundice. The belief expressed by Wangensteen is that the bleeding tendency does not hinge on decreased coagulability. According to Linton, determination of the coagulation time preoperatively is of little value in the prognosis of postoperative bleeding in obstructive jaundice. Snell, Greene and Rowntree found that following the production of obstructive jaundice in dogs there was in general fairly close parallelism between the degree of retention of bile and the coagulation time, although in some animals spontaneous reduction in the coagulation time was noted late in the progress of the condition.

The degree of jaundice, as demonstrated by the values for serum bilirubin, is of importance in regard to the hemorrhagic tendency according to Walters[39, 40] and Judd. Although it is well known that hemorrhage may be manifest in jaundiced patients with low values for serum bilirubin, it is also acceded that bleeding is anticipated more when the value for serum bilirubin is high.

Values for protein and viscosity of the serum are also considered in this work. Snell and Greene noted that following prolonged obstruction of the biliary tract, experimentally produced in dogs, there was progressive reduction in the proteins from 7.1 to 5.4 gm. for each 100 c.c. of serum. In regard to the viscosity of the serum, Groedel and Hubert found no parallelism between the speed of sedimentation of the corpuscles and viscosity of the serum.

Calcium of the blood has been extensively studied in jaundice. Despite earlier work to the contrary, Vines asserted, in 1921, that the presence of ionized calcium is not essential to the process of clotting. In studies of calcium made on jaundiced patients and experimental animals, Snell and Greene found no significant disturbance either in the amount or the proportion of diffusible calcium in the serum in the presence of jaundice. There was no correlation between either the total or

the diffusible calcium content of the serum, and either the serum bilirubin or the delay in the coagulation time of the blood in jaundice. They concluded that "jaundice does not produce a clinically significant disturbance in either the quantity or condition of the calcium in the serum of adult dogs or hospital patients." Because of these thorough studies, blood calcium was not considered in this work.

The duration of the jaundice is thought by many to have significance in regard to the tendency to bleeding. Wangensteen has noted that in most instances in which the bleeding has come under observation together with abnormalities of coagulation, the biliary obstruction has existed for some time. Of 58 of Petren's patients who died because of postoperative hemorrhage, 50 had jaundice for three weeks or more. Judd and Walters[39, 40] also expressed the belief that there is a relationship between the duration of the jaundice and the hemorrhagic tendency. Mayo-Robson noted increased liability to bleeding when the obstruction is due to carcinoma.

HYPOTHESES REGARDING THE ETIOLOGY OF THE HEMORRHAGIC TENDENCY

Many hypotheses have been expounded in regard to the etiology of the hemorrhagic tendency in jaundice. Retention of bile is thought by some to be a factor. Wangensteen expressed the belief that retention of bile probably is responsible for the bleeding. Snell, Vanzant, and Judd have found no correlation between the coagulation time and the values for bile acids or bilirubin. They considered, also, lipoid substances retained in the blood in jaundice but did not believe them to be etiologic. Destruction of hepatic tissue and diminution of hepatic function consequent on biliary obstruction might be considered the chief etiologic factors, according to Wangensteen. Disturbance of calcium metabolism is suggested by the work of Walters and others. Pawlow found that in dogs with complete biliary fistulas osteoporosis frequently developed. Seidel found the same to occur in some cases of biliary fistula. McCrudden noted that osteoporosis developed with continued biliary obstruction. These facts indicate that there may be some disturbance of metabolism of calcium in jaundice. On the contrary, are the findings of Vines, of Ravdin, Riegel and Morrison, and of Snell and Greene, which are equally convincing. The studies of Ravdin, Riegel and Morrison suggest a disturbance of metabolism of carbohydrate. On the administration of glucose intravenously or by stomach tube to jaundiced experimental animals they noted marked reduction in the coagulation time of the blood of each animal. Of four of six jaundiced patients the coagulation time was reduced by administration of glucose. They suggested that glucose may produce its effect by its reparative action on the liver. Partos and Svec reported exact parallelism between the coagulation time and the content of blood sugar. In the experience of Ravdin, Riegel and Morrison the coagulation time and the values for blood sugar do not consistently parallel each other, although reduction in the coagulation time is frequently associated with an increase in blood sugar. Delayed coagulation of the blood often is blamed for the hemorrhagic tendency. However, it is known that hemorrhage may occur in jaundice in the presence of normal coagulation time. Snell, Vanzant, and Judd suggested that hemorrhage in jaundice may be due to excess of heparin (antiprothrombin) in the blood, which may be produced by injury to the liver, or which may be due to decreased destruc-

tion by the liver of heparin formed elsewhere. However, since there is no method for determining heparin quantitatively in the blood, this cannot be proved.

OBSERVATIONS

Our study concerned 46 patients with jaundice; also, it included 21 patients without jaundice or hepatic disease who could properly be considered as controls. All determinations were made preoperatively. One or two of each of the various tests were done on each patient.

In this series, values for blood fibrin were found to be increased in jaundice in the majority of cases (Tables I, II, and III). Thirty-four of 43 jaundiced patients (79 per cent) had values for blood fibrin above the upper limits of our normal ranges. We could not demonstrate any definite relationship between the blood fibrin and the coagulation time. Mann, Bollman, and Markowitz had similar results. No correlation was seen between the blood fibrin, and the value for serum bilirubin. The content of fibrin of the blood revealed no relationship to the leucocyte count or to the values determined by hematocrit.

A fairly constant relationship was seen between the values for blood fibrin and the sedimentation rate of erythrocytes. This had been previously noted by many workers. The sedimentation rate was found to be increased in jaundice, and no difference was noted among the various types of jaundice. Of our 46 jaundiced patients, 43 (93.4 per cent) had sedimentation rates above normal (Tables I and II). Normal values are given in Table III. The sedimentation rate was influenced by an abnormal decrease in the number of erythrocytes, in the percentage of hemoglobin, or in the hematocrit values. This has been noted frequently by others. There was no simple relationship between sedimentation rate and the values for serum bilirubin. No relationship could be demonstrated between the sedimentation rate and the coagulation time, or the sedimentation rate and the leucocyte count.

A study of the values for protein and viscosity of the serum revealed nothing of significance, and there was no relationship between them and other factors of the blood. However, Snell and Greene found that in jaundice of long duration in experimental animals, elevation of the values for serum bilirubin became less pronounced, and the values for serum protein were decreased. The values for serum protein were decreased, also, in cases of severe malnutrition. Although these patients had lost varying amounts of weight, the reduction was not sufficient to cause changes in the values for serum protein.

Of the 46 jaundiced patients, 20 (43.5 per cent) gave evidence of a hemorrhagic tendency demonstrated by purpura, petechiae, epistaxis, blood in the stools, bleeding from the gums, vomiting of blood, or the finding of blood in the peritoneal cavity. From the standpoint of sex it is interesting to note that the 7 patients with purpura were females.

Six of the 10 patients (60 per cent) whose obstruction was due to carcinoma had a tendency to bleed. This is a higher percentage than was seen in the cases of obstructive jaundice due to other causes and would indicate that in cases of carcinoma the tendency to hemorrhage is greater. Mayo-Robson expressed this same belief. However, the number of our cases was much too small to warrant positive conclusions.

CASES WITH JAUNDICE AND WITH HEMORRHAGIC TENDENCY

CASE	SEX	DIAGNOSIS	DURATION OF JAUNDICE	MANIFESTATIONS OF HEMORRHAGE	ERYTHROCYTES, MILLIONS	HEMOGLOBIN, PER CENT	BILIRUBIN, MG. IN EACH 100 C.C. OF SERUM*	COAGULATION TIME		FIBRIN, MG. IN EACH 100 C.C.		SEDIMENTATION RATE		HEMATOCRIT		PROTEIN OF SERUM, GM.	VISCOSITY OF SERUM
								MINUTES	SECONDS	WHOLE BLOOD	PLASMA	MILLIMETERS	PER CENT	OXALATE, PER CENT	HEPARIN, PER CENT		
1	M	Carcinoma of pancreas	3 weeks	Postoperative bleeding	4.46	55	26.7	11	30			15.0	25.9	37.8	42.2	7.5	2.1
2	M	Carcinoma of gallbladder	2 weeks	Seen at necropsy	4.48	69	12.0	9		522	855	32.5	60.0	39.0	46.0	8.6	2.0
3	M	Carcinoma of pancreas	4 weeks	Blood in stools	3.07	48	11.0			559	825			32.2		5.8	1.9
							11.6	5	45	550	792	50.5	78.9	30.5	36.0	6.8	2.0
							11.6	6								6.5	1.4
4	F	Carcinoma	5 weeks	Purpura, bleeding from gums	2.00	36	16.8	11		477	627	70.3	91.2	23.9	23.0	6.4	1.9
							16.2	14	35	469	642	65.6	90.2	27.0	27.3		
5	F	Carcinoma	5 weeks	Epistaxis, purpura, petechiae, blood in stools	4.50	73	28.4	12		471	748	38.0	70.0	37.0	45.5	8.2	2.2
										501	645	49.0	71.0	22.4	30.5		
6	F	Carcinoma of gallbladder	9 weeks	Purpura	3.80	60	23.2	7		407	652	47.0	81.0	37.5	42.0	7.5	
							20.0	8		295	435			32.1			
7	M	Stricture of common bile duct	6 months	Blood in stools, postoperative bleeding	3.86	63	13.4	9		435	670	44.0	76.0	35.0	42.0	8.2	2.2
8	M	Stricture of common bile duct	1 month	Epistaxis	3.16	50		9				61.0	88.0		31.0		
9	F	Stricture of common bile duct	8 months	Purpura	4.10	65	5.2	8	35	328	540	48.0	78.3	39.1	38.7		
10	F	Stricture of common bile duct	5 months	Postoperative bleeding	4.40	72	21.2	10		440	687	36.5	64.6	36.0	43.5	7.8	2.2
11	F	Stricture of common bile duct	2 years	Vomiting of blood	2.82	50	11.3	16	30	551	725	58.0	91.0	24.0	36.0	7.5	2.4
12	F	Stone in common bile duct	3 months	Postoperative bleeding	3.64	65	10.3	10	30	301	437	34.5	58.0	31.0	40.5	7.3	2.0
								12	30								
13	F	Stricture of common bile duct, biliary cirrhosis	14 months	Purpura	3.71	68	13.0	11		588	852	44.0	73.0	31.0	40.0	6.7	2.0
14	F	Stricture of common bile duct	4 weeks	Purpura	3.28	55	21.1	15		574	870	32.0	55.0	34.0	42.0	7.0	2.0
15	F	Stone in common bile duct	4 weeks	Purpura	3.06	52	17.6	6	30	619	950			34.8			
					3.50	49	22.4	6		403	670	35.3	61.0	39.8	42.2	8.1	2.2
16	F	Chronic cholecystitis with cholelithiasis	7 weeks	Purpura	4.92	76	1.3	8	30	464	690	59.2	86.5	32.7	31.6		
17	F	Stricture of common bile duct, biliary cirrhosis	2 months	Purpura, epistaxis	4.37	60	27.0			386	690	42.0	75.0	44.0	44.0	7.3	2.0
					4.94	78	23.0	7	40	413	750	35.0	63.6	44.8	45.0	8.1	2.2
18	F	Subacute yellow atrophy of liver	1 week	Petechiae	4.60	72	39.0	11	30	369	561	27.5	46.5	34.3	41.0	6.9	2.0
19	F	Intrahepatic jaundice	1 year	Purpura	4.03	68	3.9	8	30	254	440	48.8	81.3	42.1	40.0		
							4.8	8	30	216	370	48.9	82.8	41.4	41.0	8.1	2.4
20	F	Portal cirrhosis	1 month	Purpura	4.46	68	31.2	6		349	590	33.2	59.6	40.8	44.3		

*van den Bergh reaction direct.

TABLE II

CASES WITH JAUNDICE BUT WITHOUT HEMORRHAGIC TENDENCY

CASE	SEX	DIAGNOSIS	DURATION OF JAUNDICE	ERYTHROCYTES, MILLIONS	HEMOGLOBIN, PER CENT	BILIRUBIN, MG. IN EACH 100 C.C. OF SERUM*	COAGULATION TIME		FIBRIN, MG. IN EACH 100 C.C.		SEDIMENTATION RATE		HEMATOCRIT		PROTEIN OF SERUM, GM.	VISCOSITY OF SERUM
							MINUTES	SECONDS	WHOLE BLOOD	PLASMA	MILLIMETERS	PER CENT	OXALATE, PER CENT	HEPARIN, PER CENT		
21	M	Carcinoma of biliary ducts	3 months	4.19	70	18.8	9	30	417	613	47.2	76.5	32.0	38.2	7.0	2.0
22	M	Carcinoma of pancreas	1 month	1.72	16	10.0	14		500	588	85.0	94.0	15.0	10.0	6.6	2.0
23	M	Carcinoma of pancreas	6 weeks	4.61	75	43.9	6	30	452	637	30.0	46.0	29.0	35.0	6.5	1.9
24	M	Carcinoma of pancreas	2 weeks	4.16	66	15.5	8				28.0	46.0	37.0	39.0	6.6	1.9
25	M	Acute cholecystitis with stones	3 days	4.60	70	2.1	9		623	1060	42.0	80.7	41.2	48.0		
						2.3			767	1252	50.0	85.9	38.7	41.8		
26	M	Chronic cholecystitis	1 week	4.00	70	4.3	7	30	298	505	48.0	81.3	40.9	41.0	8.7	2.0
						2.0	6		326	575	36.0	61.0	43.2	39.0	7.6	2.2
27	M	Cholecystitis with cholelithiasis	1 week	4.30	70	3.6	7		354	655	39.1	72.1	45.9	45.8	8.3	2.2
28	M	Chronic cholecystitis with cholelithiasis	12 days	4.10	70	6.0	10	30	687	1007	39.2	72.6	31.8	46.0	7.5	2.3
29	M	Stricture of common bile duct	5 weeks	4.32	72	5.8	11	30	421	712	33.5	60.2	41.0	44.5	7.5	2.0
30	M	Subacute cholecystitis; cholangeitis; stone in common bile duct	4 months	4.10	65	2.2	10		531	805	43.0	77.0	34.0	44.0	9.1	2.3
31	M	Subacute cholecystitis; stone in common bile duct	3 years	3.67	65	5.6	7		479	807	37.3	67.8	40.6	45.0	8.4	
									307	650	22.5	45.0	52.7	50.0	8.7	2.1
						3.5	7	30	421	745	18.0	36.0	43.4	50.0		
32	M	Biliary cirrhosis with cholelithiasis	8 days	5.01	80	14.6	8		426	765	1.0	1.8	44.2	45.0		
33	M	Stricture of common bile duct	4 days	3.71	66	7.5	6	30	321	547	43.0	74.1	41.2	42.0		
34	M	Subacute cholecystitis with cholelithiasis, obstruction of common bile duct		5.02	80	2.4	9		359	680	26.5	51.1	47.1	48.2		
35	F	Stone in common bile duct	10 days	3.79	50	4.5	11	30	391	697	45.1	77.7	43.9	42.0	7.8	2.1
						2.4	8	30	452	765	49.1	81.8	40.8	40.0		
36	F	Stricture of common bile duct	11 months	4.00	65	5.5	8		332	575	46.3	80.6	42.2	42.6	7.9	2.1
						4.7	6		281	480	48.1	80.3	41.3	40.1		

*van den Bergh reaction direct.

TABLE II (CONTINUED)

CASE	SEX	DIAGNOSIS	DURATION OF JAUNDICE	ERYTHROCYTES, MILLIONS	HEMOGLOBIN, PER CENT	BILIRUBIN, MG. IN EACH 100 C.C. OF SERUM*	COAGULATION TIME		FIBRIN, MG. IN EACH 100 C.C.		SEDIMENTATION RATE		HEMATOCRIT		PROTEIN OF SERUM, GM.	VISCOSITY OF SERUM
							MINUTES	SECONDS	WHOLE BLOOD	PLASMA	MILLIMETERS	PER CENT	OXALATE, PER CENT	HEPARIN, PER CENT		
37	F	Stone in common bile duct	7 weeks	3.80	58	20.2	13		600	845	53.2	84.5	29.0	37.0	8.4	2.2
38	F	Stricture of common bile duct	2 weeks	2.92	59	3.0	9	30	1188	1650	57.0	87.0	28.0	34.5	8.4	2.1
39	F	Stone in common bile duct	8 weeks	2.77	50	9.0	8		742	990	60.0	91.0	25.0	34.0	5.9	1.8
40	F	Stone in common bile duct	10 weeks	3.52	65	4.9	8		510	785	53.0	84.0	35.0	37.0	7.0	2.1
41	F	Stricture of common bile duct	1 week	3.70	58	5.6	7		581	932	49.1	80.4	37.6	39.0	7.6	2.0
						5.2	7	30	546	862	50.8	83.2	36.0	39.0	7.5	2.1
42	F	Stricture of common bile duct	10 days	3.85	65	22.4	8	30	635	1115	44.8	80.0	43.0	44.0	9.0	2.6
						22.6	8	30			52.0	86.2	39.7		8.3	2.3
						23.0	9	30	589	1007	54.3	86.1	41.5	37.0		
43	M	Intrahepatic jaundice	5 weeks	4.01	65	22.2	11	30	605	878	42.0	76.3	31.0	45.0	8.2	2.1
44	F	Cirrhosis of liver	6 years	2.50	39	7.5	7	30	378	525	62.2	89.4	28.0	30.5	8.6	2.2
						7.6	10	30	408	585	63.0	89.7	30.2	29.8	7.8	2.3
45	F	Intrahepatic jaundice	3 weeks	3.71	65	37.5	8	30	243	397	42.6	69.4	38.8	38.7		
46	F	Intrahepatic jaundice	1 year	3.75	45	3.0	7	30	675	945	52.0	79.5	28.5	34.6	8.5	2.3
				3.70	48	2.9	8		443	657	51.0	77.2	32.6	34.0	9.3	2.5

TABLE III
CASES WITHOUT JAUNDICE

CASE	SEX	DIAGNOSIS	ERYTHROCYTES, MILLIONS	HEMOGLOBIN, PER CENT	BILIRUBIN, MG. IN EACH 100 C.C. OF SERUM*	COAGULATION TIME		FIBRIN, MG. IN EACH 100 C.C.		SEDIMENTATION RATE		HEMATOCRIT		PROTEIN OF SERUM, GM.	VISCOSITY OF SERUM
						MINUTES	SECONDS	WHOLE BLOOD	PLASMA	MILLIMETERS	PER CENT	OXALATE, PER CENT	HEPARIN, PER CENT		
1	M	Normal						364	660						
2	M	Benign hypertension	5.20	78				242	450	11.8	22.6	46.1	48.0		
3	M	Thromboangiitis obliterans						364	660			44.7	43.0		
4	M	Diabetes mellitus	4.50	70				304	580	14.3	26.4	47.5	46.0		
5	M	Anxiety neurosis	4.42	75				241	472	13.0	23.8	48.8	45.4		
6	M	Hypertension; arteriosclerosis	4.56	70				392	680	12.0	21.8	42.3	45.0	6.5	1.9
7	M	Arteriosclerosis; diabetes mellitus	5.15	75	1.7	7		326	635	10.5	21.6	48.6	51.5	7.7	2.0
8	M	Duodenal ulcer	4.98	70	2.4	8	10	225	420	2.5	4.6	46.2	46.0		
9	M	Phlebitis	3.99	65	2.2	8		282	537	17.9	33.7	47.5	47.0	6.9	1.9
10	M	Neurosis; pylorospasm; latent syphilis	5.09	82	2.3	7		282	532	5.5	10.5	46.9	48.0	8.0	2.0
11	M	Hyperinsulinism	4.96	78		6	20	417	790	39.8	73.0	47.2	45.5		
12	M	Gastric ulcer	4.56	80	1.1	6	30	319	575	12.0	21.8	44.4	45.0		
13	M	Duodenal ulcer	4.58	68	2.4	5		239	515	4.0	8.3	53.4	52.0	8.2	2.1
14	M	Thromboangiitis obliterans	5.20	82	1.1	6	30	458	825	20.2	36.9	44.4	45.3		
15	M	Senile dementia	4.62	80	2.2	6		342	627	7.8	14.9	45.4	47.7		
16	M	Duodenal ulcer	4.40	62	0.8	11		173	287	6.0	10.0	39.5	40.0	7.2	1.8
17	F	Normal			2.3			223	415	10.0	18.5	46.2	46.0		
18	F	Nervous exhaustion	4.20	70	1.2	8		280	495	3.0	5.5	43.0	45.4		
					1.4	6	30	246	457	15.0	27.6	46.1	45.7		
19	F	Ventral hernia; chronic cholecystitis with cholelithiasis	4.69	80	1.7	8	30	361	697	29.2	58.0	48.1	49.7	8.7	2.5
20	F	Diabetes mellitus; chronic cholecystitis	5.11	75				318	505	26.0	41.9	37.0	38.0		
21	F	Chronic cholecystitis with cholelithiasis	4.28	60	0.3	8	30	419	685	36.5	62.9	38.7	42.0		
22	F	Leiomyoma of uterus; bilateral ovarian cystadenoma; chronic salpingitis	4.87	80	0.9	5		344	580	16.1	26.9	40.6	40.3		

*van den Bergh reaction indirect.

In the cases in which the tendency to bleeding was demonstrated, the jaundice was usually of longer duration than in the other cases. Seventeen (85 per cent) of the former patients had jaundice of four weeks' duration or longer as compared with 14 (54 per cent) of the latter. This observation agrees with that of Petren, Walters, Judd and others.

The values for serum bilirubin were somewhat higher in the cases in which there was bleeding of some type. Of these, in 52.6 per cent the values for bilirubin in each 100 c.c. of serum were 15 mg. or more, whereas, among the other cases of jaundice, similar values were found in only 26.9 per cent.

The coagulation time was found to be somewhat longer in cases in which there was a hemorrhagic tendency than in the other cases of jaundice. In 25 per cent of the former and in 7.7 per cent of the latter, the coagulation time was twelve minutes or more.

Values for blood fibrin were increased above normal in the greater number of cases of jaundice. The values were slightly higher in those cases in which there was no bleeding, as compared with those in which there was a tendency to bleeding.

Study of the sedimentation rate of erythrocytes revealed nothing significant. Four of our patients bled postoperatively and three of these had sedimentation rates higher than normal. However, the sedimentation rate was increased to more than normal in 41 other cases in which there was no postoperative bleeding (Tables I, II, III). This increase in the sedimentation rate is due in part to the effects of the increase in blood fibrin in many of the cases, and of the anemia which frequently occurs in jaundice.

Study of the erythrocyte count, percentage of hemoglobin and hematocrit readings in regard to the tendency to bleeding disclosed no significant relationship. Studies of the values for protein and viscosity of the serum were equally negative.

From our study, several interesting facts have been noted regarding the hemorrhagic tendency in jaundice. The content of blood fibrin could not be shown to be at fault in this condition. The values for blood fibrin in the cases in which there was a hemorrhagic tendency were slightly less than those in which there was no evidence of hemorrhage, but the majority were still higher than normal. Although Linton found the sedimentation test of prognostic value in regard to the possibility of bleeding, we should hesitate, from our results, to ascribe any importance to it. In considering the possibility of hemorrhage among jaundiced patients, the value for serum bilirubin and the duration of the jaundice must be considered, as well as the coagulation time.

CONCLUSIONS

Within the limits of the data presented, the deviations from the normal of the blood fibrin, sedimentation rate of erythrocytes, and protein and viscosity of the serum are not sufficiently great to be of significance in explaining the hemorrhagic tendency in jaundice. From the clinical standpoint the duration of the jaundice, its intensity as measured by the elevation in serum bilirubin and the prolongation of the coagulation time are of greater importance in judging the possibility of postoperative hemorrhage in the individual patient.

REFERENCES

1. Bircher, M. E.: Clinical Diagnosis by the Aid of Viscosimetry of the Blood and Serum With Special Reference to the Viscosimeter of W. R. Hess, J. Lab. & Clin. Med. **7**: 134-147, 1921.
2. Fåhraeus, Robin: The Suspension Stability of the Blood, Physiol. Rev. **9**: 241-274, 1929.
3. Foster, D. P.: A Clinical Study of Blood Fibrin With Observations in Normal Persons, Pregnant Women, and in Pneumonia and Liver Diseases, Arch. Int. Med. **34**: 301-312, 1924.
4. Foster, D. P., and Whipple, G. H.: Blood Fibrin Studies: I. An Accurate Method for the Quantitative Analysis of Blood Fibrin in Small Amounts of Blood, Am. J. Physiol. **58**: 365-378, 1921.
5. Gram, H. C.: The Sedimentation of the Blood-Corpuscles in Various Internal Diseases and the Result of Correction of This Value for the Variations of the Hemoglobin Percentage, Acta med. Scandin. **70**: 242-275, 1929.
6. Greisheimer, Esther M.: The Blood Sedimentation Test in Normal Men and Women, Am. J. M. Sc. **174**: 338-343, 1927.

7. Greisheimer, Esther M., Johnson, Olga H., and Ryan, Mary: The Relationship Between Sedimentation Index and Fibrin Content in Relatively Normal Individuals, Am. J. M. Sc. **177**: 816-827, 1929.
8. Greisheimer, Esther M., Ryan, Mary, and Johnson, Olga H.: The Relationship Between Cell Count and Sedimentation Index, Am. J. Physiol. **89**: 170-175, 1929.
9. Groedel and Hubert: Quoted by Baer, J. L., and Reis, R. A.: Further Studies in Sedimentation, Am. J. Obst. & Gynec. **12**: 740-744, 1926.
10. Hubbard, R. S., and Geiger, H. B.: Anemia as a Factor in the Sedimentation Time of Erythrocytes, J. Lab. & Clin. Med. **13**: 322-326, 1927.
11. Hunt, H. F.: Studies of Sedimentation of Erythrocytes, J. Lab. & Clin. Med. **13**: 327-333, 1927.
12. Hunt, H. F.: Studies of Sedimentation of Erythrocytes, J. Lab. & Clin. Med. **14**: 1061-1077, 1929.
13. Judd, E. S.: Surgical Procedures in Jaundiced Patients, J. A. M. A. **85**: 88-91, 1925.
14. Lee, R. I., and White, P. D.: A Clinical Study of the Coagulation Time of Blood, Am. J. M. Sc. **145**: 495-503, 1913.
15. Linton, R. R.: The Sedimentation Rate of Blood as an Index of the Haemorrhagic Tendency in Obstructive Jaundice, Ann. Surg. **91**: 694-704, 1930.
16. Mann, F. C., Bollman, J. L., and Markowitz, J.: A Study of the Relation of the Liver to the Process of Coagulation of the Blood, Am. J. Physiol. **90**: 445, 1929.
17. Mayo-Robson, A. W.: The Surgery of the Gall Bladder and Bile Ducts, With Brief Notes of 78 Cases, Brit. M. J. **1**: 901-904, 1894.
18. McCrudden, F. H.: Quoted by Wangensteen.
19. Neuhausen, B. S., and Rioch, D. M.: The Refractometric Determination of Serum Proteins, J. Biol. Chem. **55**: 353-356, 1923.
20. Partos, A., and Svec, F.: Quoted by Radvin, Riegel and Morrison.
21. Pawlow, J. P.: Quoted by Wangensteen.
22. Petren, G.: Quoted by Wangensteen.
23. Pinner, Max, Knowlton, Kathryn, and Kelly, Ruby G.: The Sedimentation Rate of Erythrocytes. Its Relation to Fibrin Value and Cholesterol Content and Its Application in Tuberculosis, Arch. Path. & Lab. Med. **5**: 810-827, 1928.
24. Plass, E. D., and Rourke, M. D.: A New Procedure for Determining Blood Sedimentation Rates, J. Clin. Investigation **5**: 531-539, 1928.
25. Ravdin, I. S., Riegel, Cecelia, and Morrison, J. L.: Coagulation of Blood. I. The Comparative Values of Calcium and Glucose as Agents for Decreasing the Clotting Time, Ann. Surg. **91**: 801-810, 1930.
26. Rosenthal, N., and Blowstein, M. I.: The Sedimentation Time of Blood in Jaundice, J. Lab. & Clin. Med. **14**: 464-472, 1929.
27. Rourke, M. D., and Plass, E. D.: An Investigation of Various Factors Which Affect the Sedimentation Rate of the Red Blood Cells, J. Clin. Investigation **7**: 365-386, 1929.
28. Rubin, E. H., and Smith, N. N.: Relation of Hemoglobin, Cell Count and Cell Volume to the Erythrocyte Sedimentation Reaction, Arch. Int. Med. **39**: 303-314, 1927.
29. Schultz, E. W., Nicholes, J. K., and Schaefer, J. H.: Studies on Blood Fibrin. Its Quantitative Determination; Normal Fibrin Values, and Factors Which Influence the Quantity of Blood Fibrin, Am. J. Path. **1**: 101-115, 1925.
30. Seidel, H.: Quoted by Wangensteen.
31. Snell, A. M., and Greene, C. H.: The Calcium in the Serum in Jaundice, Am. J. Physiol. **92**: 630-638, 1930.
32. Snell, A. M., Greene, C. H., and Rowntree, L. G.: Diseases of the Liver. II. A Comparative Study of Certain Tests for Hepatic Function in Experimental Obstructive Jaundice, Arch. Int. Med. **36**: 273-291, 1925.
33. Snell, A. M., Vanzant, Frances R., and Judd, E. S.: The Complications and Sequelae of Prolonged Obstructive Jaundice, Med. Clin. N. Am. **13**: 1417-1438, 1930.
34. Starlinger, W.: Quoted by Rosenthal and Blowstein.
35. van den Bergh, Hijmans: Jaundice, Brit. M. J. **2**: 498-500, 1924.
36. Vines, H. W. C.: The Coagulation of the Blood. Part I. The Rôle of Calcium, J. Physiol. **55**: 86-99, 1921.
37. Walters, Waltman: Preoperative Preparation of Patients With Obstructive Jaundice, Surg. Gynec. Obst. **33**: 651-656, 1921.
38. Walters, Waltman: II. Preoperative Preparation of Patients With Obstructive Jaundice: End Results in Thirty-Four Cases, Minnesota Med. **6**: 25-28, 1923.
39. Walters, Waltman: Clinical Application of Studies in Obstructive Jaundice, Wisconsin M. J. **28**: 150-156, 1929.
40. Walters, Waltman: Obstructive Jaundice: Its Treatment, Complications and Results of Treatment, New England J. M. **201**: 1-6, 1929.
41. Wangensteen, O. H.: The Haemorrhagic Diathesis of Obstructive Jaundice and Its Treatment, Ann. Surg. **88**: 845-865, 1928.

STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM*

XIX. The Effect of Diet on Urinary Acid and Ammonia Excretion in Man

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THE literature on ammonia metabolism is so large that one hesitates to present further data. However, there is surprisingly little in regard to normal ammonia excretion with diets of known acidity and with known nitrogen metabolism. This paper presents such data.

EXPERIMENTAL PROCEDURE AND PRECAUTIONS

The series of individuals (summarized in the addenda) studied in the research ward of the Massachusetts General Hospital were maintained on a continuously unvaried diet for periods of two weeks to three months. The routine methods used have been described in detail elsewhere.[1] Each patient was kept on a preliminary adjustment period of from three to six days: even so, a few observations have been excluded because it was obvious that a steady state had not been attained by the end of this time. Although the data here summarized comprise analyses (in duplicate) of over 400 twenty-four-hour specimens on nine patients, only the values attained at a steady state or "metabolic equilibrium" are given.

Successive specimens of urine from each subject were iced as soon as voided and kept, stoppered, with chloroform, until the end of each twenty-four-hour period, when titratable acidity (modified after Henderson and Palmer[2]) and ammonia (Folin[3]) were determined on the mixed twenty-four-hour specimen. Most of the ammonia analyses were made by the permutit-nesslerization method, but a considerable number were also made by the aeration procedure and satisfactory checks obtained.

In a few instances a relatively high ammonia concentration was encountered in an alkaline urine. It seemed desirable, therefore, to compare in an extreme case (patient DB) the summated acidity and ammonia content of individual specimens (analyzed as soon as passed) with the values later determined for the accumulated twenty-four-hour specimen. The results in Table I exclude the possibility of bacterial or chemical action (outside of the body) producing the high ammonia content and alkaline reaction; and justify the technic employed.

WHAT IS THE URINARY ACID AND AMMONIA EXCRETION OF A HUMAN ADULT ON A POTENTIALLY NEUTRAL DIET?

In Table II are given the figures for several patients, each value representing three days' excretion, i. e., the sum of three consecutive daily determinations. The

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Received for publication, April 30, 1932.
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TABLE I

PATIENT DB—TETANY, ACCOMPANYING STEATORRHEA.
ANALYSIS OF INDIVIDUAL AND COMBINED TWENTY-FOUR-HOUR SPECIMENS OF URINE

DAY EXCRETED	TIME EXCRETED	WHEN ANALYZED	VOLUME C.C.	ACID C.C. N/10	AMMONIA C.C. N/10
Wednesday	11:20 A.M.				
	9:00 A.M.	Wednesday	315	-25	72
		Thursday		-28	71
	1:00 P.M.	Wednesday	135	- 6	43
	7:30 P.M.	Wednesday	235	25	160
	10:00 P.M.	Wednesday	100	8	61
Thursday	12:30 A.M.	Thursday (1:00 A.M.)	555	15	85
	9:00 A.M.	Thursday	470	-67	124
		Friday (5:00 P.M.)		-63	124
Total Calculated Mixed Specimen		Thursday (10:00 A.M.)	1810	-49 -56	545 530

diets were planned to be potentially neutral according to data on foodstuffs used in this laboratory.* In the table the potential acidity of the diet is also shown, when recalculated according to Sherman (1927).[5] The average ammonia excretion on such nearly neutral diets amounts to approximately 210 c.c. N/10 daily.

It is interesting to compare these values with those of Henderson and Palmer,[6] and with the results of Blatherwick.[7] The first of these investigators took no special precautions with regard to the potential acidity of the diet of their subjects; and the last used phenolphthalein as the reference indicator for titration. Their results, therefore, as recalculated in Table III for three-day periods, tend to be higher than ours. We have no data, unfortunately, to enable us to calculate the actual acid-base content of Blatherwick's basal diet, which yielded nearly neutral urines and approximately the same titratable acidities as ours.

REPRESENTATIVE LEVELS OF URINARY ACID AND AMMONIA EXCRETION AT VARYING LEVELS OF ACID-INTAKE

For purposes of clinical metabolic research, it was essential to establish standard levels of human urinary ammonia excretion at various levels of ingested acid. Such levels may be attained either by change in diet or by feeding alkali- or acid-producing electrolyte. The results shown in Fig. 1 demonstrate the marked rise (roughly logarithmic) in ammonia excretion with increasing potential acidity of the diet.

The actual equilibrium values involved are given in Table IV.

THE EFFECT OF ABSORPTION BY BOWEL

Variations of acid-base balance within the bowel may complicate the total acid-base balance, and particularly the urinary excretion. Salter, Farquharson, and

*The reliability of the extant data for calculating acidities of diets has been discussed elsewhere by Salter, Fulton, and Angier.[4]

TABLE II

URINARY ACID EXCRETION (THREE-DAY PERIODS†) ON "NEUTRAL DIET"

SUBJECT‡	TITRATABLE ACIDITY $-CO_2$ C.C. N/10	AMMONIA C.C. N/10	TOTAL ACID C.C. N/10	POTENTIAL ACIDITY OF DIET CALCULATED FROM SHERMAN, 1927, C.C. N/10	POTENTIAL ACIDITY OF DIET CALCULATED FROM DATA OF THIS LABORATORY C.C. N/10 FOR 3 DAYS	NITROGEN BALANCE GRAMS PER 3 DAYS
WN	193	551	744	− 75	30	3.3
	192	555	747			3.0
	531	692	1223			−1.3
RN	416	598	1014	−141	24	−0.5
	526	635	1161			−8.3
AN-I*	−617	1132		−153	10	2.9
	−635	1201				4.6
AN-II*	95	637	732	−153	10	2.6
	− 62	526				2.7
	196	724	920			−0.4
	255	660	915			−3.2
DA	529	861	1390			−2.8
	543	744	1287	−129	4	−4.6
	434	563	997			3.8
ST	568	624	1192			4.5
	660	507	1167	−129	4	1.0
	491	371	862			6.7
LZ-II	40	579	619	39	−120	0
	93	561	654			−1.4
LZ-XVIII*	−422	532				8.1
	− 41	616				6.3
	−268	488		−165	− 26	10.0
	−267	455				8.0
DB*	−158	1732				3.4
	−400	1637		−210	10	4.3
	156	2030	2186			3.3
	−134	1876				4.6
BE	318	445	763			1.0
	177	362	539	−242	17	1.9
	257	468	725			2.7
R. F. F.	290	854	1144	− 36	− 9	−1.7
	259	750	1009			0.9

†Figures given for three-day periods are the sums of three consecutive daily determinations.

‡A brief description of each of these subjects is given in the addenda.

*In alkaline urines the sum of titratable acidity and ammonia is omitted because of its questionable significance unless CO_2 is also measured.

Tibbetts[8] studied the neutralization of ingested sodium acid phosphate by bowel and kidney, respectively. They pointed out that the kidney could compensate for loss of base (through the bowel) by increase in ammonia production. This efficient base-conservation by the kidney was shown by Gamble, Blackfan and Hamilton[9] to be the usual result of the administration of acid-producing salts (like calcium chloride). Such high ammonia excretion is, of course, usually accompanied by high urinary acidity. It cannot be assumed, however, that this is invariably true.

Of special interest is patient DB, whose minimal level of ammonia excretion was much higher than that of other patients of this series, even though the urine was frequently alkaline in reaction. This patient suffered intermittently from mild tetany due to deficient fat assimilation. It is unlikely, however, that tetany per se was responsible for the high ammonia excretion: because a low ammonia was obtained with patient WR, who had parathyroid tetany of several years' standing.[10]

TABLE III

URINARY ACIDITY VS. EXCRETION OF TOTAL ACID
(CALCULATED FOR THREE-DAY PERIODS)

	TITRATABLE ACIDITY $-CO_2$ C.C. N/10	AMMONIA C.C. N/10	TOTAL ACID C.C. N/10
Henderson and Palmer[6]			
$P_H = 5.4$	960	1101	2061
$P_H = 6.6$	672	1071	1743
Blatherwick[7]			
Subject B	271	221	
	266	226	
	284	208	
	821	655	1476
Subject W	253	169	
	286	193	
	264	204	
	803	566	1369

TABLE IV

MINIMAL AND MAXIMAL URINARY AMMONIA PRODUCTION (PER THREE DAYS)

SUBJECT	NEUTRAL DIET WITH ADDED $NaHCO_3$		NEUTRAL DIET	HIGH ACID DIET		NEUTRAL DIET WITH ADDED NH_4Cl	
	POTENTIAL ACIDITY OF DIET C.C. N/10	URINARY AMMONIA EXCRETION C.C. N/10	URINARY AMMONIA EXCRETION C.C. N/10	POTENTIAL ACIDITY OF DIET C.C. N/10	URINARY AMMONIA EXCRETION C.C. N/10	POTENTIAL ACIDITY OF DIET C.C. N/10	URINARY AMMONIA EXCRETION C.C. N/10
DA	−2103	242	563	2111	1389	6736	3948
	−7622	293	511	2170	1359	6736	4787
ST	−2138	100	507	2171	1227	6736	4737
	−7304	73	371	2171	1433	6736	6131
AN-I	−4715	170	1132	2069	2136	4142	3834
	−5190	142	1201	2069	2549	3376	3982
DB*	−4532	846	2030			3366	4470
	−4615	785	1876			3366	4438
WR†			728				

*Tetany, accompanying steatorrhea.
†Tetany, idiopathic, presumably hypoparathyroidism.

It is quite possible, on the other hand, that the steatorrhea in this case was directly responsible for the high urinary ammonia. The subject lost an unusual amount of fixed base (in the form of soap) by feces; and was presumably forced to make extraordinary demands upon devices which conserve fixed base, e. g., ammonia-production.

The mechanism at work here is presumably quite similar to that of the acid-producing salts. The essential differences are first, that the acid involved is an organic acid (derived from neutral fat); and second, that there is no rise in urinary acid because there is no exogenous acid radicle to be excreted. That such excessive loss of fixed base may produce a "relative acidosis" has been shown by several investigators, whose work has been summarized by Shohl.[11]

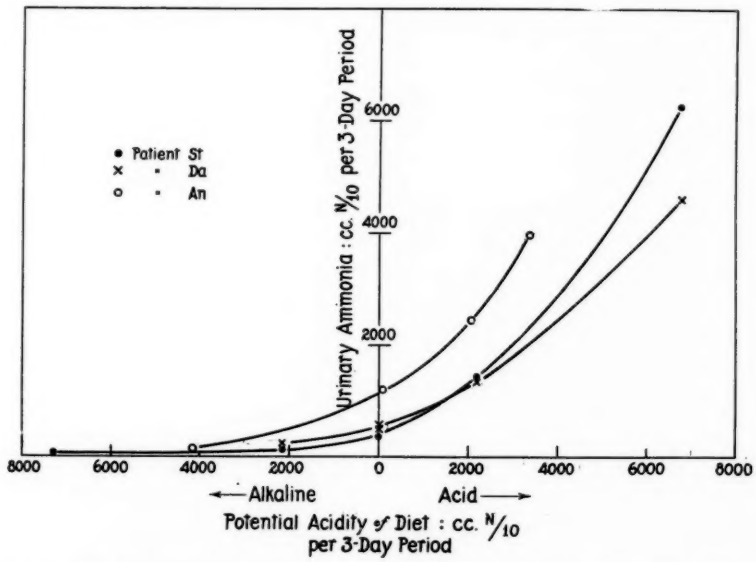

Fig. 1.—Urinary ammonia increases when potential acidity of the diet is increased.

In Table V is contrasted the fixed base excretion of patient DB with that of two normal young adults. The titratable alkalinity* of the fecal ash is also shown. Patient DB's fixed base excretion (by bowel) is high, and the titratable ash over 50 per cent of it. When calcium chloride is added to the diet, the situation is further exaggerated: because calcium is held in the bowel by excess of fatty acid. In the latter instance, titratable ash approximately equals total base.

The high urinary ammonia output in case DB may be regarded as pathologic but other similar figures were obtained with subject AN (see Table II, AN I), who when taking a potentially neutral diet, excreted considerable amounts of ammonia in an alkaline urine. It is noteworthy that the feeding of large amounts of acid-producing substances (including ammonium chloride) to this subject resulted in so great an increase in ammonia excretion that the urine remained alkaline even under the stress of eliminating a great excess of acid radicles.[12] Curiously enough, after a vacation of six months, this patient returned to the former ward and identical diet, but failed to excrete an alkaline urine (see Table II, AN II). Such excre-

*Titratable ash was determined by titrating the dry ash with HCl to methyl red.

TABLE V

RELATION OF URINARY ACID EXCRETION TO INORGANIC CONSTITUENTS OF FECES (THREE-DAY PERIODS)

SUBJECT	DIET	URINARY			TOTAL FIXED BASE OF INGESTA C.C. N/10	FECAL	
		TITRATABLE ACIDITY – CO_2 C.C. N/10	AMMONIA C.C. N/10	TOTAL ACID C.C. N/10		TOTAL FIXED BASE C.C. N/10	TITRATABLE ASH C.C. N/10
AD Normal	Control	384	835	1219	5142	1000	296
	Control	300	836	1136	5142	670	382
	Added NaCl	337	922	1259	10272	760	392
	Added NaCl	383	984	1367	11982	765	386
	Added NaCl	111	1186	1297	12257	690	382
CW Normal	Control	584	983	1567	5142	930	382
	Control	570	951	1521	5142	1120	385
	Added NaCl	421	1007	1428	10275	940	368
	Added NaCl	536	990	1411	11939	1060	423
	Added NaCl	353	1174	1427	12840	870	290
DB Tetany, Accompanying Steatorrhea	Control	65	2065	2130	5175	2530	1380
	Added $CaCl_2$	401	3543	3944	10035	3980	4250

tion values serve to emphasize our lack of knowledge concerning ammonia excretion in health. They present no explanation for the preferential conservation of base by ammonia-production rather than by titratable acidity.

LAG IN URINARY EXCRETION FOLLOWING CHANGE IN DIET

Peters and Van Slyke[13] have commented upon the sluggish response of ammonia excretion to the ingestion of acid. In order to determine the length of time which must elapse following a dietary change before a steady metabolic state is attained, daily determinations were made until constant excretion-values were approached. From a survey of Fig. 2 and similar data obtained from the subjects listed in Table II it seems obvious that equilibrium values may not be reached in less than five to seven days after a dietary change. It is true, however, that the excretion of urinary titratable acidity reaches equilibrium level earlier than other factors, such as calcium and ammonia excretion.

Fig. 2 indicates, also, how constant the acid-excretion of a single individual may become on a constantly continued metabolic regimen when once a plateau-level of excretion has been attained.

EFFECT OF NITROGEN BALANCE ON URINARY ACID EXCRETION

From inspection of the nitrogen balances listed in Table II, it appears that the subjects studied were for the most part in nitrogen equilibrium, or retained a surplus of nitrogen. The chief exception is subject RN, who in one period apparently used 8 gm. of body nitrogen. Estimating the potential acidity of one gram of human protein-nitrogen as 44 c.c. of N/10 acid (see data for meat given by Sher-

man[5]) this negative nitrogen balance might conceivably account for 366 c.c. of N/10 acid during the three days in question. As one surveys the apparently spontaneous variations which occur in total acid excretion, one is led to believe that the excretions given in Table II may be regarded as essentially independent of the nitrogen balance.

This impression is substantiated, if one studies specifically the effect of two common acid-producing agents: i. e., protein and ammonium chloride. As a crucial test, we studied the effect of these substances on ammonia excretion when sufficient sodium bicarbonate was fed (simultaneously) to balance their potential acidity.

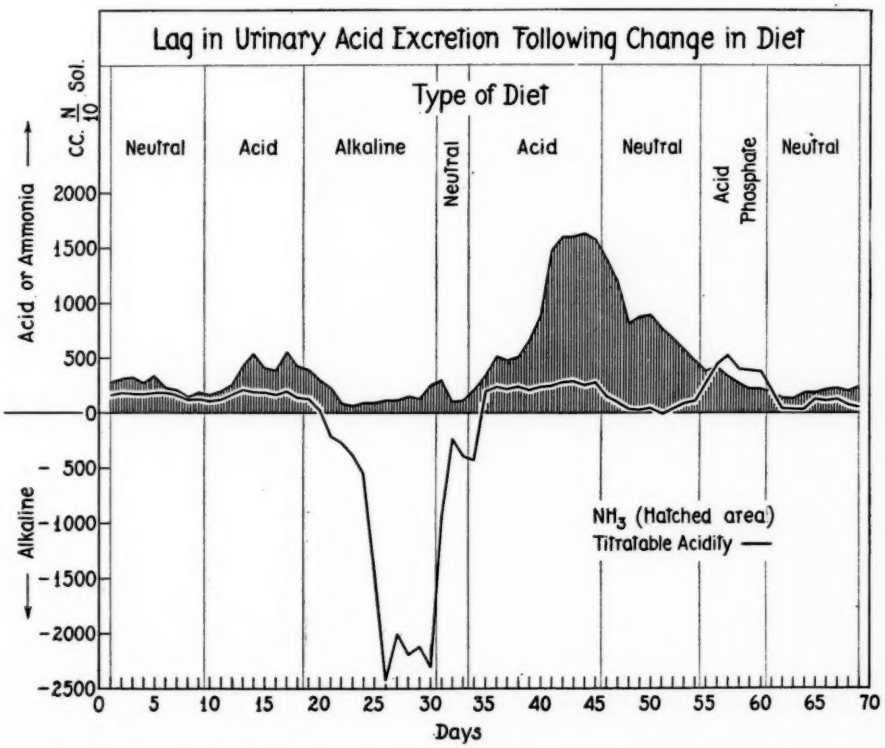

Fig. 2.—Excretion of acid in consecutive twenty-four-hour specimens of urine during variation in the potential acidity of the diet. The term "diet" is here used to designate all ingesta, including both food and medication. The daily potential acidity of the respective "diets" indicated in the figure are (in sequence):
Neutral = 0. Acid = 724 c.c. N/10. Alkaline = 714 c.c. N/10 for the first 6 days, 2600 c.c. N/10 thereafter.
Neutral = 0. Acid = 738 c.c. N/10 for the first 5 days, 2266 c.c. N/10 thereafter.
Neutral = 0. Acid phosphate = 713 c.c. N/10.
(For description of subject see DA in addenda.)

THE EFFECT OF INGESTED NEUTRALIZED PROTEIN ON AMMONIA EXCRETION

In order to determine whether the ingestion of large amounts of protein per se affected the formation of urinary ammonia, a normal adult was fed protein to the extent of 202 gm. daily after a control test on a neutral diet containing 58.6 gm. of protein per day. Simultaneously with the high-protein diet sufficient $NaHCO_3$ was fed to hold the titratable acidity at its former level. Under these circumstances, only a slight (possibly negligible) rise in ammonia was observed. When alkali was

discontinued, a marked increase in urinary ammonia (as well as titratable acidity) occurred (see Table VI). These results indicate that the effect of a high-protein diet in increasing urinary ammonia is due mainly to its acid catabolites. They show that when such protein is neutralized the resulting ammonia excretion progressively approaches a low plateau level. They minimize the importance, therefore, both of the nitrogen balance, when positive, and of the urinary nitrogen excretion (per se) as prime factors in determining urinary ammonia.

TABLE VI

EFFECT OF INGESTED PROTEIN ON URINARY AMMONIA PRODUCTION
(THREE-DAY PERIODS)

SUBJECT R.F.F.

	POTENTIAL ACIDITY OF DIET C.C. N/10	$NaHCO_3$ ADDED GRAMS	POTENTIAL ACIDITY OF TOTAL INGESTA C.C. N/10	URINARY	
				TITRATABLE ACIDITY – CO_2 C.C. N/10	AMMONIA C.C. N/10
Low Protein, Neutral Diet	–9		–9	521 290 259	934 854 750
High Protein Diet, Neutralized by $NaHCO_3$	3387	27 26	233 352	–27 50	928 1088
High Protein Diet, plus Excess $NaHCO_3$	3387	90	–7323	–7259	226
High Protein Diet, without $NaHCO_3$	3387		3387	1568	2240

TABLE VII

EFFECT OF INGESTED AMMONIA ION ON URINARY AMMONIA
(THREE-DAY PERIODS)

SUBJECT AN-II

	POTENTIAL ACIDITY OF DIET C.C. N/10	$NaHCO_3$ ADDED GRAMS	POTENTIAL ACIDITY OF TOTAL INGESTA C.C. N/10	TITRATABLE ACIDITY – CO_2 C.C. N/10	AMMONIA C.C. N/10
Control, Neutral diet	–7 10			95 – 62	638 526
Neutral Diet plus NH_4Cl (6 grams daily)	10		3334 3376	702 654	1953 2637
Neutral Diet plus NH_4Cl (6 grams daily) plus $NaHCO_3$	10	37 29 23	–511 –135 698	–683 –297 286	492 558 613

AMMONIA INGESTION VS. AMMONIA EXCRETION

The possibility that ingested NH_4 ion (administered in the form of NH_4Cl) might contribute to urinary ammonia has been commented upon by Gamble, Blackfan, and Hamilton.[9] In order to control our neutralized protein experiment, sub-

ject AN, after attaining equilibrium on a nearly neutral diet, was given 6 gm. of NH_4Cl daily. Subsequently, $NaHCO_3$ was also given (alternately with each dose of NH_4Cl) in sufficient quantity to produce a nearly neutral urine. The results are in agreement with a similar experiment of Haskins[14] who fed ammonium carbonate directly.

THE INFLUENCE OF PHOSPHATE EXCRETION ON URINARY AMMONIA

The reason for the increase in urinary ammonia following the ingestion of unneutralized protein is not clear. One might on first thought suspect the increased elimination of phosphoric acid (from oxidized protein) as the causative agent. The influence of acid phosphate on the elimination of ammonia in the urine has been discussed by Marriott and Howland,[15] however, who pointed out that increase in

TABLE VIII

EFFECT OF PHOSPHATE INGESTION ON URINARY AMMONIA AND TITRATABLE ACIDITY (THREE-DAY PERIODS)

SUBJECT	DIET	PHOSPHORUS IN FOOD GM.	TOTAL PHOSPHORUS INGESTED GM.	POTENTIAL ACIDITY OF INGESTA C.C. N/10	TITRATABLE ACIDITY—CO_2 C.C. N/10	AMMONIA C.C. N/10	TOTAL ACID C.C. N/10
DA	Neutral control diet (moderate phosphorus)	2.2		4	543	744	1287
		2.2		4	434	563	997
	Acid diet	2.8		2111	585	1389	1974
		2.9		2170	508	1359	1867
	Neutral diet plus NaH_2PO_4	2.2	10.1	2142	1179	699	1878
WN	Neutral control diet	1.9			193	551	744
		1.9		31	192	555	747
	Control diet plus NaH_2PO_4	1.9	10.8	2428	1222	757	1979
		1.9	10.8	3235	870	776	1646
	Control diet immediately after acid phosphate	1.3		102	381	956	1337
		1.9		− 18	141	914	1055
		1.9		31	362	932	1294
LZ	Neutral diet, moderate phosphorus	2.1		− 66	−121	608	
		2.1		− 83	− 4	608	
	High equimolecular phosphate	2.1	5.4	268	−263	541	
		2.1	5.4	324	114	525	639
			5.4	268	70	362	432
	Control diet	1.8		24	531	692	1223
		1.8		24	416	598	1014
		1.2		− 13	526	635	1161
	Control diet plus Na_2HPO_4	1.8	8.5	−284	70	306	376
		1.8	8.7	−348	−397	313	293
		1.8	8.4	−328	17	276	
	Control diet	1.6		− 96	305	416	721
		1.9		− 8	180	428	608
	Control diet plus NaH_2PO_4	1.9	9.0	1962	1008	697	1705
		1.9	8.9	2025	878	573	1451

phosphate excretion spares ammonia-production. Similarly, Haldane[16] found that ingestion of 30 gm. of $NaH_2PO_4.2H_2O$ produced no fall in alveolar CO_2, nor rise in urinary ammonia although the acidity of the urine rose to 0.114N. This effect is not due to any peculiar suppressing influence of the phosphate radicle per se upon ammonia production, but rather is a logical result of the relation of urinary phosphate to acid excretion by the kidney.[8]

In Table VIII are presented data on total acid excretion by individuals who were fed acid or alkaline phosphate. The results indicate very little change in ammonia excretion despite marked variation in excretion of titratable acid. They substantiate, therefore, the findings of previous investigators; and are presented only because they represent quantitative excretion-levels in human beings on known intakes. They point to other acidic groups, e. g., sulphur, in protein as the cause of increased ammonia excretion.

SUMMARY

In human adults on a potentially neutral diet, urinary ammonia amounts to about 210 c.c. N/10 daily.

When the need for acid elimination is controlled (by adjusting the potential acidity of the food metabolized), the urinary ammonia excretion of human beings is found to increase in a roughly logarithmic fashion as more acid is fed.

Without recourse to inorganic acids (or acid-producing salts), it is difficult to attain a normal ammonia excretion above 800 c.c. N/10 daily, because the phosphate in most acid-producing foods tends to relieve urinary ammonia production.

The urinary response to acid or basic diets is complicated by (1) variations in base-excretion by the gastrointestinal tract and (2) by lags in the renal base-conserving mechanisms (i. e., ammonia and titratable acidity).

High protein intake does not increase ammonia production, provided that simultaneously enough alkali is administered to neutralize its acid catabolites.

ADDENDA

DESCRIPTION OF PATIENTS

A brief description of each of the patients (listed in Table III) used in this investigation follows:

WN: A married female of thirty-four years of age, weighing 89 kilos, suffering from chronic atrophic arthritis of two years' duration.

RN: A single female of thirty-nine years of age, weighing 56 kilos, suffering from rheumatic heart disease (mitral stenosis), chronic bronchitis, and bronchial asthma. During the period of observation she had no cough, her chest was clear, and there were no signs of myocardial failure.

AN: A male, aged sixteen, weighing 34 kilos, suffering from marked structural scoliosis of spine. Admission I—at rest in supporting plaster shell. Admission II —six months after a spinal fusion for correction of his deformity, followed by a vacation.

DA: A male aged thirty-seven, weighing 58 kilos, recovering from lead poisoning. Mild extensor paresis of upper extremities (fingers and wrists). No colic during periods presented here.

ST: A male, aged forty-six, weighing 51 kilos, recovering from lead poisoning. No paralysis. No colic during periods presented here.

LZ: A male, aged eighteen, weighing 60 kilos, recovering from chronic multiple neuritis; confined to bed because of weakness of extremities, but feeling well.

DB: A female, aged twenty-seven, weighing 43 kilos, suffering from tetany associated with deranged fat absorption for some four years. Free from tetany at time of this investigation, but serum calcium was only 5.6 mg. per 100 c.c.

BE: A male, aged fifty-eight, weighing 55 kilos, suffering from chronic sciatic neuritis of ten months' duration.

RFF: A male, aged thirty, weighing 78 kilos, healthy, normal, a physician.

REFERENCES

1. Bauer, W., and Aub, J. C.: Studies of Inorganic Salt Metabolism. I. The Ward Routine and Methods, J. Am. Dietetic Assn. **3**: 106, 1927.
2. Henderson, L. J., and Palmer, W. S.: On the Several Factors of Acid Excretion, J. Biol. Chem. **17**: 305, 1914.
3. Folin, O.: Laboratory Manual of Biological Chemistry, 1925, Appleton, 119.
4. Salter, W. T., Fulton, C., and Angier, F.: Studies in Calcium and Phosphorus Metabolism. XI. The Calculation of Acid-Base Content of the Diet, J. Nutrition **4**: 1, 1931.
5. Sherman, H. C., Chemistry of Food and Nutrition, 1927, Macmillan.
6. Henderson, L. J., and Palmer, W. W.: On the Several Factors of Acid Excretion, J. Biol. Chem. **17**: 310, 1914, Table II.
7. Blatherwick, N. R.: The Specific Role of Foods in Relation to the Composition of Urine, Arch. Int. Med. **14**: 409, 1914.
8. Salter, W. T., Farquharson, R. F., and Tibbetts, D. M.: Studies of Calcium and Phosphorus Metabolism, XIV. The Relation of Acid-Base Balance to Phosphate Balance Following Ingestion of Phosphates, J. Clin. Invest. **11**: 391, 1932.
9. Gamble, J. L., Blackfan, K. D., and Hamilton, B.: A Study of the Diuretic Action of Acid-Producing Salts, J. Clin. Invest. **1**: 359, 1924-5.
10. Aub, J. C., Albright, F., Bauer, W., and Rossmeisl, E.: Studies of Calcium and Phosphorus Metabolism, VI. In Hypoparathyroidism and Chronic Steatorrhea with Tetany with Special Consideration of the Therapeutic Effect of Thyroid, J. Clin. Invest. **11**: 211, 1932.
11. Shohl, A. T.: Mineral Metabolism in Relation to Acid-Base Equilibrium, Physiological Reviews **3**: 519, 1923.
12. Farquharson, R. F., Salter, W. T., Tibbetts, D. M., and Aub, J. C.: Studies of Calcium and Phosphorus Metabolism. XII. The Effect of the Ingestion of Acid-producing Substances, **10**: 226, 1931, Table I.
13. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, **1**: 1931, Williams and Wilkins Co.
14. Haskins, H. D.: Nitrogenous Metabolism as Affected by Diet and by Alkaline Diuretics, J. Biol. Chem. **2**: 271, 1906-07.
15. Marriott, W. McK., and Howland, J.: The Influence of Acid Phosphate on the Elimination of Ammonia in the Urine, Arch. Int. Med. **22**: 477, 1918.
16. Haldane, J. B. S.: Experiments on the Regulation of Blood Chemistry II, J. Physiol. **55**: 265, 1921.

VARIATIONS IN CERTAIN CONSTITUENTS OF THE BLOOD OF RELATIVELY NORMAL INDIVIDUALS*†

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THIS study was undertaken because of the lack of agreement among authors in regard to the composition of the blood. Attention will be directed to two constituents only, although equally great discrepancies are found in many others. A comprehensive review will not be attempted.

Hammett (1920) in sixty determinations on nine individuals, found a range of 27.3 to 45.5, with an average of 35.6 mg. per cent for total nonprotein nitrogen; for urea nitrogen, he found the limits to be 9.7 and 23.8, with an average of 17.1 mg. per cent. In his series, the urea nitrogen comprised 47.8 per cent of the total nonprotein nitrogen.

Berglund (1922) studied twelve normal subjects, in whom he found a range of 27.8 to 39.4, with an average of 32.1 mg. per cent; the urea nitrogen limits were 8.9 and 15.2, with an average of 11.5 mg. per cent; in this series, the urea nitrogen formed 35.82 per cent of the total. This author stated that in wealthy subjects, on a high protein diet, he found the total nonprotein nitrogen to be between 40 and 45 mg. per cent.

Myers (1924) gave the range of total nonprotein nitrogen as 25 to 35 and of urea, 12 to 15 mg. per cent; he states that the urea nitrogen forms 50 per cent of the total nonprotein nitrogen.

Mathews (1925) gave the range as 26 to 43 for total nonprotein nitrogen, and 10 to 22 for urea nitrogen.

Hawk and Bergeim (1927) gave 25 to 30 mg. per cent for the total, and stated that the urea nitrogen forms 50 per cent of the total nonprotein nitrogen.

Bodansky (1930) gave the range as 30 to 35 for total nonprotein nitrogen, and 14 to 20 for urea nitrogen, and stated that the urea nitrogen forms 50 to 60 per cent of the total.

In view of such discrepancies, it was decided to study several constituents of the blood in a large group of men and women. The individuals who volunteered to act as subjects were members of the faculty, students, and parents or relatives of students. All subjects were in comparatively good health, engaged in various types of work, and free from known disease; consequently, they were considered relatively normal.

The group comprised 105 men and 109 women. The mean age of the men was 25.65 years, of the women, 29.27 years, and of the group as a whole, 27.49 years. The age range for the men was 18 to 63 years, and for the women 18 to 77 years.

The blood (20 c.c.) was drawn by venipuncture between seven and eight o'clock in the morning, after a twelve to fourteen hour fast. Only those cases in

*From the Department of Physiology, The University of Minnesota.
Received for publication, June 17, 1932.
†A grant from the Medical Research Fund helped to defray the cost of this research.

which a complete analysis of the seven selected constituents was accomplished were considered in the statistical treatment of the results.

The following seven constituents were determined, by the methods designated:

1. True glucose-Shaffer-Somogyi (1929).
2. Serum calcium-in duplicate-Clark-Collip (1925) modification of Kramer-Tisdall (1921).
3. Inorganic phosphate-Fiske-Subbarow (1925).
4. Serum chloride-Whitehorn (1920-21).
5. Urea nitrogen-direct Nesslerization of the unlaked blood filtrate, Folin (1930 b), after incubation of the buffered whole blood with urease paper, Folin (1930 a).
6. Total nonprotein nitrogen-Koch-McMeekin (1924) oxidation and direct Nesslerization of the tungstate-sulphuric acid filtrate, Folin (1919).
7. Uric acid-Benedict (1922).

Table I presents the means found for the men, women, and group as a whole, and the range of each constituent.

TABLE I

CONSTITUENT	MEANS (MG. PER CENT) MEN	WOMEN	COMBINED	RANGE COMBINED
Sugar	83.95 ± 0.47	84.07 ± 0.45	84.01 ± 0.33	59.65 – 101.59
Calcium	10.581 ± 0.020	10.601 ± 0.026	10.591 ± 0.016	9.69 – 12.15
Inorganic phosphate	3.433 ± 0.027	3.406 ± 0.029	3.419 ± 0.020	2.58 – 4.90
Sodium chloride	574.4 ± 1.0	579.6 ± 1.0	577.1 ± 0.72	535 – 609
Urea nitrogen	13.56 ± 0.19	13.49 ± 0.19	13.53 ± 0.13	7.5 – 23.8
Total nonprotein nitrogen	34.87 ± 0.23	32.98 ± 0.26	33.91 ± 0.18	21.3 – 44.1
Uric acid	3.417 ± 0.034	3.070 ± 0.029	3.240 ± 0.024	2.10 – 5.52

The urea nitrogen formed 39.90 per cent of the total nonprotein nitrogen.

Table II presents the standard deviations of each constituent.

TABLE II

CONSTITUENT	STANDARD DEVIATIONS MEN	WOMEN	COMBINED
Sugar	7.18 ± 0.33	6.99 ± 0.32	7.08 ± 0.23
Calcium	0.305 ± 0.014	0.399 ± 0.018	0.356 ± 0.012
Inorganic phosphate	0.415 ± 0.019	0.449 ± 0.020	0.433 ± 0.014
Sodium chloride	15.63 ± 0.73	15.71 ± 0.72	15.65 ± 0.51
Urea nitrogen	2.84 ± 0.13	2.93 ± 0.13	2.884 ± 0.094
Total nonprotein nitrogen	3.57 ± 0.17	4.05 ± 0.18	3.94 ± 0.13
Uric acid	0.522 ± 0.024	0.456 ± 0.021	0.520 ± 0.017

Table III presents the coefficients of variation of each constituent.

TABLE III

CONSTITUENT	COEFFICIENTS OF VARIATION MEN	WOMEN	COMBINED
Sugar	8.55	8.31	8.43
Calcium	2.88	3.76	3.36
Inorganic phosphate	12.09	13.18	12.67
Sodium chloride	2.72	2.71	2.71
Urea nitrogen	20.92	21.71	21.32
Nonprotein nitrogen	10.23	12.28	11.61
Uric acid	15.28	14.87	16.04

The differences between the means for the men and women are given in Table IV. The means for the women are used as the subtrahends.

TABLE IV

CONSTITUENT	DIFFERENCES BETWEEN MEN AND WOMEN DIFFERENCE	DIFFERENCE/ERROR
Sugar	−0.12 ±0.65	0.18
Calcium	−0.020 ±0.033	0.61
Inorganic phosphate	+0.027 ±0.039	0.69
Sodium chloride	−5.2 ±1.4	3.7
Urea nitrogen	+0.07 ±0.27	0.26
Total nonprotein nitrogen	+1.89 ±0.35	5.4
Uric acid	+0.347 ±0.045	7.7

We were interested in determining whether or not a relationship existed between certain pairs of constituents. This was studied by means of the correlation coefficients. The results are presented in Table V.

TABLE V

CORRELATION COEFFICIENTS	
Urea—total nitrogen (nonprotein)	+0.542 ±0.032
Sugar—phosphate	−0.089 ±0.046
Sugar—calcium	+0.045 ±0.046
Calcium—phosphate	+0.134 ±0.045

The relationship between urea nitrogen and total nonprotein nitrogen is the only one of significance.

SUMMARY AND CONCLUSIONS

Seven constituents of the blood have been studied on 214 (105 men and 109 women) relatively normal individuals in a fasting condition.

The age range was eighteen to seventy-seven years, with an average of 27.49 years.

The mean for true glucose is 84.01±0.33; this agrees well with previous work by this method (Holt and Greisheimer, 1931, and Glassberg, 1931). The difference between the sexes is not significant. The true glucose level bears no relation to the level of either calcium or inorganic phosphate; this is shown by the low correlation coefficients.

The mean serum calcium is 10.591±0.016; the difference between the sexes is not significant. The present values are slightly lower than those found earlier (Greisheimer, 1929), but the difference between the two series is of doubtful significance.

The mean inorganic phosphate is 3.419±0.020, with no significant difference between the sexes. The inorganic phosphate level bears no relation to the serum calcium level, as shown by the absence of correlation.

The mean serum sodium chloride is 577.06±0.72, with no significant difference between the sexes.

The mean urea nitrogen is 13.53±0.13, with no significant difference between the sexes; it formed 39.90 per cent of the total nonprotein nitrogen; it bears a defi-

nite relation to the total, as would be expected, and as is shown by the high degree of correlation.

The total nonprotein nitrogen is 33.91±0.18; the mean for the men is significantly higher than that for the women.

The mean uric acid is 3.240±0.024; the mean for the men is significantly higher than that for the women.

REFERENCES

Benedict, S. R.: The Determination of Uric Acid in Blood, J. Biol. Chem. **51**: 187, 1922.

Berglund, H.: Nitrogen Retention in Chronic Interstitial Nephritis and Its Significance, J. A. M. A. **79**: 1375, 1922.

Bodansky, M.: Introduction to Physiological Chemistry, 1930.

Clark, E. P., and Collip, J. B.: A Study of the Tisdall Method for the Determination of Blood Serum Calcium With a Suggested Modification, J. Biol. Chem. **63**: 461, 1925.

Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, J. Biol. Chem. **66**: 375, 1925.

Folin, O., and Wu, H.: A System of Blood Analysis, J. Biol. Chem. **38**: 81, 1919.

Folin, O., and Svedberg, A.: An Improved Distillation Method for the Determination of Urea in Blood, J. Biol. Chem. **88**: 77, 1930 (a).

Folin, O., and Svedberg, A.: Micro Methods for the Determination of Nonprotein Nitrogen, Urea, Uric Acid, and Sugar, in Unlaked Blood, J. Biol. Chem. **88**: 85, 1930 (b).

Glassberg, B. Y.: The Blood Sugar of Normal Fasting Persons, Proc. Soc. Exper. Biol. & Med. **28**: 889, 1931.

Greisheimer, E. M.: The Relationship Between Serum Calcium and Age, Am. J. M. Sc. **177**: 704, 1929.

Hammett, F. S.: Studies of Variations in the Chemical Composition of Human Blood, J. Biol. Chem. **41**: 599, 1920.

Hawk, P. B., and Bergeim, O.: Practical Physiological Chemistry, 1927.

Holt, G. W., and Greisheimer, E. M.: "True" Glucose Tolerance in Forty-Two Normal Individuals, Proc. Soc. Exper. Biol. & Med. **28**: 547, 1931.

Koch, F. C., and McMeekin, T. L.: A New Direct Nesslerization Microkjeldahl Method and a Modification of the Nessler-Folin Reagent for Ammonia, J. Am. Chem. Soc. **46**: 2066, 1924.

Kramer, B., and Tisdall, F. F.: A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum, J. Biol. Chem. **47**: 475, 1921.

Mathews, A. P.: Physiological Chemistry, 1925.

Myers, V. C.: Practical Chemical Analysis of Blood, 1924.

Shaffer, P., and Somogyi, M.: Improved Methods for Blood Sugar Determinations, Directions Distributed at the Thirteenth International Physiological Congress, 1929.

Whitehorn, J. C.: A System of Blood Analysis; Supplement II; Simplified Method for the Determination of Chlorides in Blood or Plasma, J. Biol. Chem. **45**: 449, 1920-1921.

STUDIES IN THE SEROLOGY OF SYPHILIS*

XI. A Clinical and Statistical Evaluation of a New Flocculation Test for Syphilis: Based on 26,611 Tests in Comparison With the Wassermann Reaction, and 2473 in Comparison With the Kahn

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PRECIPITATION tests for syphilis are handicapped by two inherent difficulties: (1) the large personal factor involved in the reading of weak positive reactions, in which the aggregates are difficult to detect and (2) the multiplicity of variables which must be rigidly controlled in order to obtain consistent or even reliable results, variables which have been discussed in detail elsewhere.† These two difficulties are masked by the apparent technical simplicity of the reaction, and only come to light when, upon analyzing the results in comparison with the Wassermann reaction, one uncovers a high incidence of false positive or false negative results.

In an attempt to eliminate, or at least decrease the importance of these two disturbing factors, the author recently devised a precipitation test,** embodying (1) an optimum adjustment of the variables which control the aggregation, insofar as these could be ascertained on empirical and theoretical grounds: (2) the use of the centrifuge to facilitate aggregation; and (3) the use of a new sensitizing substance, corn germ sterol, as an adjunct to cholesterol. The advantages of the test were stated to be (1) a greater sensitivity: (2) simplicity of technic, as no titrations are necessary, and the test involves the use of only one tube; (3) an increased reliability, due to the ease of reading results, and the wide margin of safety in the adjustment of the reagents: (4) convenience, as the antigen dilution keeps for at least three days, and the incubation time can be varied from one-half hour, for emergency tests, to as long as twenty-four hours, for maximal sensitivity, with four hours as the routine incubation period; (5) cheapness, one cubic centimeter of antigen sufficing for 50 to 100 tests.

The present paper is an analysis of the results obtained with the test at the Johns Hopkins Hospital during the period August 10, 1931, to April 1, 1932. They are presented as statistical evidence for the sensitivity and reliability of the reaction as claimed in the first communication. The material comprises 26,611 sera of a general dispensary and hospital population, divided into two groups. The one group consists of 10,383 tests on approximately 1500 known syphilitic patients under active treatment or observation in the Syphilis Clinic of the Johns Hopkins Hospital. The remaining 16,228 tests were on approximately 14,000 patients in the remainder of the dispensary and in the general hospital. This group included 2441 tests on children under fourteen years of age, some of whom were under treatment for congenital syphilis.

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Received for publication, June 15, 1932.
†Am. J. Syph. **16**: 218, 1932.
J. Lab. & Clin. Med. **17: 787, 1932.

Every serum was tested by a four hour ice box Wassermann reaction and by the precipitation test in question. In addition, 1328 sera from Group 1 (syphilitic), and 1045 sera from Group 2 (general hospital and dispensary) were tested by the Kahn reaction. The same individual who read the Wassermann results (a laboratory technician) also read the precipitation reactions. Kahn test results were read by the author.

I. *Technic of the Various Tests.*—

1. *The New Precipitation Test.—Antigen*: Fifty grams of dried powdered beef heart (Difco) are extracted at 37° C. for fifteen minutes with 200 c.c. of ether with intermittent shaking. The process is repeated three times and the ether filtrates discarded. The powder is then dried and extracted with 250 c.c. of 95 per cent ethyl alcohol for three to five days at 37° with repeated shaking. The extract is then filtered and the moist powder washed with 3 to 50 c.c. portions of 95 per cent alcohol. The combined filtrate and washings are evaporated down to 250 c.c. One and a half grams cholesterol and 1.5 grams corn germ sterol are then added and dissolved by boiling, a final concentration of 0.6 per cent for each. The corn germ sterol can be obtained from the Digestive Ferments Company, Detroit.

Technic of Test: The antigen is heated at 55° to 75° C. for a few minutes to dissolve all of the sensitizer. One and three-tenths volumes of 4 per cent NaCl are blown into 1 volume of antigen, and the milky suspension allowed to "ripen" for at least thirty minutes at room temperature. Kept in the ice box, this suspension is good for forty-eight to seventy-two hours, gradually increasing in sensitivity. Our routine procedure was to prepare the dilution twenty-four hours in advance. This routine "aging" has since been prolonged to forty-eight hours.

The sera to be tested are inactivated at 56° C. for twenty minutes. To 0.4 c.c. are added 0.04 c.c. of the antigen dilution, the tubes shaken for two minutes, and placed at 37° C. for four hours. At the end of this time, the tube is centrifuged for fifteen minutes at 1500 revolutions per minute, and 1.2 c.c. of 0.85 per cent NaCl then added.

In a strongly positive serum, there is seen a very coarse aggregation of the antigen with a perfectly surrounding clear fluid. In a less strongly positive serum there are definite aggregates. A negative serum remains opalescent, with a homogeneous cloud of refractile particles. Any intermediate result, in which the contents of the tube seem finely granular, lacking the watered-silk appearance of the negative reaction, or in which there are a few nondescript particles, is read as doubtful. All such doubtful results are recentrifuged at higher speed after shaking; if the test is really positive, the antigen forms a coherent white flake at the bottom of the tube. The re-centrifugation of any doubtful result is essential for best results.

Reports are made as positive, negative, or doubtful.

2. *Wassermann Reaction.—Antigen**: Fifty grams of dried powdered beef heart (Difco) are extracted with 250 c.c. of ether for fifteen minutes at 37° C. The ether filtrate is discarded. The moist powder is washed on the filter with 100 c.c.

*As will be shown in a later paper, it has since been found that the precipitation antigen can be used in the Wassermann test as well, giving identically the same results as the Wassermann antigen used in this series. For use in the Wassermann test, the precipitation antigen is diluted 1:100, prepared by dropping two volumes of salt solution slowly into one volume of antigen, and then pouring in the remaining 97 volumes of salt solution.

of warm ether, dried, and extracted at 37° C. for four days with 250 c.c. of 95 per cent ethyl alcohol. After filtration, the moist powder is washed with 100 c.c. of alcohol and the combined alcoholic filtrate and washings evaporated down to 75 c.c. Seventy-five cubic centimeters of absolute alcohol are then added. The extract is then sensitized with 0.8 per cent cholesterol and 0.6 per cent sitosterol (8 and 6 mg. per c.c. respectively), which are dissolved by boiling. The sitosterol can be obtained from the Digestive Ferments Company, Detroit.

Technic of Test: The clear serum is inactivated at 56° C. for twenty minutes, and 0.1, 0.1, and 0.05 c.c. respectively placed in three tubes. Complement is prepared by pooling the blood of at least 4 guinea pigs, allowing it to clot overnight at ice box temperature, and centrifuging. Two-tenths cubic centimeter of a 1:12 dilution in 0.85 per cent NaCl are added to all three tubes.

The antigen is heated at 56° C. for a few minutes to redissolve excess sensitizer, and diluted by slowly dropping one volume of antigen, with shaking, into 200 volumes of 0.85 per cent salt solution.* Two-tenths cubic centimeter of the dilution are added to Tubes 2 and 3, Tube 1 serving as an anticomplementary control.

Two-tenths cubic centimeters of salt solution are added to Tube 1, making a total volume of 0.5, 0.5, 0.45, in the three tubes.

The tests are then placed in the ice box for four hours, followed by one-half hour incubation at 37° C. Four-tenths cubic centimeters of a sensitized cell suspension are then added and the results read after fifteen to twenty minutes at 37° C.

The sensitized cells are prepared by washing citrated sheep's blood with 10 volumes of salt, and diluting the measured packed cells with 32 volumes of salt solution. The minimal dilution of amboceptor which will cause the hemolysis of this suspension in one-half hour is then determined (0.2 c.c. of amboceptor plus 0.2 c.c. per cent cells plus 0.2 c.c. 1:12 complement plus 0.4 c.c. salt solution). The 3 per cent cell suspension is then sensitized with an equal volume of amboceptor dilution containing 2½ to 3 times this minimal hemolytic quantity. Four-tenths cubic centimeter of the resultant 1½ per cent suspension are used in the test.

Reports are made as positive, negative or doubtful.

3. *Kahn Reaction.*—Antigen was obtained from Dr. R. L. Kahn, to whom I express my appreciation. The technic used is exactly that described in his book on the subject, except that only one tube was used, with a 1:8 antigen: serum ratio (0.4 serum + 0.05 c.c. of antigen dilution).

II. *Sensitivity: Results in a Known Syphilitic Population (10,383 Tests).*—

a. The results summarized in Tables I and II are self-explanatory. From 72 to 75 per cent of the sera in a general syphilitic population under active antisyphilitic treatment or observation, gave positive or doubtful precipitation tests as compared with 58.7 per cent for the Wassermann† and 60.2 per cent for the Kahn reactions. Even these figures do not indicate the degree of greater sensitivity; for of those detected by the Wassermann and Kahn, 7.7 per cent and 6.2 per cent respectively were doubtful; while a definite reading of positive or negative was made

*A more sensitive antigen dilution can be prepared by dropping 2 volumes of salt solution, slowly and with shaking, into 1 volume of antigen, and after a two- to five-minute interval, adding the remaining 198 volumes of salt solution.

†A systematic technical error (the use of unsterile citrate in the collecting of the sheep's blood) caused the cell suspension to be quite fragile over a period of at least three months. Due to this error, the figures given for the positive Wassermann reactions are at least 5 per cent too low, as will appear in a forthcoming analysis of a second series of 30,000 tests.

in all except 1.6 per cent of the precipitation tests. The large number of anticomplementary sera in the Wassermann reaction in this group was found to be due to the presence in the serum of arsenicals inadvertently introduced in the collecting tube, such specimens being particularly likely to be anticomplementary when allowed to stand at room temperature over the week-end.

The greater sensitivity of the new precipitation test over the Wassermann and Kahn reactions (and we wish to emphasize the fact that this is a comparatively sensitive Wassermann technic), becomes clearly manifest when we note that the precipitation test detected 15.3 per cent (1594 sera) missed by the Wassermann reaction entirely and 13.4 per cent missed by the Kahn: while the reverse was true in only 1.1 and 1 per cent respectively.

TABLE I

COMPARISON OF THE WASSERMANN AND PRECIPITATION TESTS IN 10,383 SERA FROM A SYPHILITIC POPULATION UNDER TREATMENT OR OBSERVATION

	ABSOLUTE AGREEMENT 72.5%			RELATIVE AGREEMENT 7%		RELATIVE DISAGREEMENT 1.7%		ABSOLUTE DISAGREEMENT 16.4%		ANTICOMPLEMENTARY 2.5%		
Wassermann	−	±	+	±	+	−	±	−	+	Anticomplementary		
Precipitation	−	±	+	+	±	±	−	+	−	−	±	+
	2335	28	5142	685	40	91	84	1594	118	54	6	206
	22.5%	0.3%	49.7%	6.6%	0.4%	0.9%	0.8%	15.3%	1.1%	0.5%	0.06%	2%

	POSITIVE	DOUBTFUL	NEGATIVE
Wassermann	5300 = 51%	797 = 7.7%	4020 = 38.7%
Precipitation	7627 = 73.4%	165 = 1.6%	2591 = 25%

TABLE II

COMPARATIVE SENSITIVITY OF THE KAHN AND AUTHOR'S PRECIPITATION TESTS IN 1328 SYPHILITIC SERA

	ABSOLUTE AGREEMENT 78.8%			RELATIVE AGREEMENT 5.2%		RELATIVE DISAGREEMENT 1.6%		ABSOLUTE DISAGREEMENT 14.3%	
Kahn	−	±	+	+	−	−	±	−	+
Precipitation	−	±	+	+	±	±	−	+	−
	344	2	701	65	4	6	16	177	13
	26.9%		52.7%	4.9%	0.3%	0.4%	1.2%	13.5%	1%

	POSITIVE	DOUBTFUL	NEGATIVE
Kahn	718 = 54%	83 = 6.2%	527 = 39.79%
Precipitation	943 = 71%	12 = 1%	373 = 28.1%

The contrast between the fine granular appearance of the positive Kahn tests, and the coarse, readily visible clumps of the precipitation reaction under discussion was usually striking. The recentrifugation of all tests about which there was the slightest doubt contributed considerably to the clarity of the results and to the comparative infrequency of doubtful reports.

As expected corollaries of this greater sensitivity, (1) patients with early syphilis under active treatment regularly continue to give a positive precipitation test for weeks and even months after the Wassermann has become negative; (2) old

cases of syphilis whose Wassermann remains weakly positive, varying between positive, negative, and doubtful from week to week, usually give a persistently positive precipitation test; (3) many cases of syphilis, placed upon probation after one or two years' treatment, their Wassermann having become and remained negative, are found to retain small quantities of reagin in their serum, the precipitation test being persistently positive.

In this series of 10,383 tests, there was absolutely no evidence that either the Wassermann or precipitation test have selective sensitivity for particular clinical conditions. Titration experiments in our hands indicate that if reagin is present in high concentration, both types of test are positive; if present in small amounts, the precipitation test may give a positive reaction when the Wassermann reaction is negative. Of 2612 tests in this group, in which the two tests disagreed, the precipitation test was more strongly positive in 2370. It is nevertheless true that a few patients were encountered in whom the Wassermann reaction was persistently positive, the precipitation test persistently negative. This phenomenon could not be attributed to a selective reactivity of the former in particular clinical conditions, for the cases belonged to no one clinical group, being a fair random sample of the entire series of cases.

b. *Results in a General Hospital and Dispensary Population (16,228 tests).*—The data summarized in Table III again illustrate the greater sensitivity of the precipitation test, as well as its comparative freedom from doubtful results. The total number of positive reactions in this general group of hospital and dispensary patients was 2117 (13 per cent), as compared with 1432 (8.9 per cent) for the Wassermann reaction. The precipitation test detected 503 positive sera in which the Wassermann was negative; the situation was reversed in only 49; while the incidence of doubtful reactions was 0.7 per cent for the precipitation test, and 1.4 per cent for the Wassermann test.

Qualitatively the same results were obtained in comparing the Kahn and precipitation tests in a small group of patients (Table IV), the Kahn test proving more sensitive than the Wassermann, but distinctly less sensitive than the precipitation test.

There were at least 46 patients with clinically active syphilis in the general hospital-dispensary group never before treated or examined in this clinic, in whom the first precipitation test was positive, while the first Wassermann was negative

TABLE III

RESULTS IN A GENERAL HOSPITAL POPULATION: 16,228 WASSERMANN AND PRECIPITATION TESTS

	ABSOLUTE AGREEMENT 93.7%			RELATIVE AGREEMENT 1%		RELATIVE DISAGREEMENT 0.9%		ABSOLUTE DISAGREEMENT 3.4%		ANTI-COMPLEMENTARY 1%		
Wassermann	−	±	+	±	+	−	±	−	+	Anticomplementary		
Precipitation	−	±	+	+	±	±	−	+	−	−	±	+
	13814	13	1372	161	11	93	58	503	49	70	3	81
	85.1%		8.5%	1%		0.6%	0.3%	3.1%	0.3%	0.4%		0.5%

	POSITIVE	DOUBTFUL	NEGATIVE
Wassermann	1432 = 8.9%	232 = 1.4%	14410 = 88.8%
Precipitation	2117 = 13%	120 = 0.7%	13991 = 86.2%

(24), doubtful (13), or anticomplementary (9). As is seen in Table V, there is again no evidence of selectivity for a particular clinical condition.

TABLE IV

RESULTS IN A GENERAL HOSPITAL AND DISPENSARY POPULATION 1045 KAHN AND PRECIPITATION TESTS

	ABSOLUTE AGREEMENT 95.8%			RELATIVE AGREEMENT 0.9%		RELATIVE DISAGREEMENT 1.3%		ABSOLUTE DISAGREEMENT 2%	
Kahn	−	±	+	±	+	−	±	−	+
Eagle	−	±	+	+	±	±	−	+	−

	POSITIVE	DOUBTFUL	NEGATIVE
Kahn	96 = 9.2%	15 = 1.4%	934 = 89.4%
Eagle	111 = 10.6%	8 = 0.8%	926 = 88.6%

TABLE V

46 CASES OF CLINICALLY ACTIVE SYPHILIS GIVING A POSITIVE PRECIPITATION TEST, AND A NEGATIVE, DOUBTFUL OR ANTICOMPLEMENTARY WASSERMANN REACTION

WASSERMANN REACTION	PRECIPITATION TEST	CLINICAL CONDITION							TOTAL
		CONGENITAL	PRIMARY OR SECONDARY	NEUROSYPHILIS	AORTITIS OR AORTIC INSUFFIC.	GUMMA	KERATITIS, IRITIS	BONE	
−	+	1	1	9	6	1	4	2	24
anticomp.	+	2	1	3	2	1			9
±	+	2	1	3	4	1	2		13

III. *Specificity of the Precipitation Test.*—

There remains to be answered the essential question of specificity. Are these numerous discrepancies between the Wassermann and precipitation tests due actually to the greater reactivity of the latter with syphilitic sera only, *or*, are they due even in part to nonspecific reactions with normal nonsyphilitic sera?

A. *Doubtful Reactions.*—As reported in this laboratory, doubtful reactions include any test in which, whether because of adventitious particles in the serum (dirt, denatured protein), dirt in the tube, or indefinite aggregation of the lipoid particles, no definite evaluation of the test in terms of positive or negative is possible. Any report of doubtful is thus not a diagnosis, but an admission on the part of the laboratory that a definite report could not be rendered, calling for a careful serologic and clinical check-up. If all subsequent tests are negative, the original doubtful report is presumably due to some technical factor. If subsequent tests prove positive, or if the patient presents clinical evidence of syphilis, the doubtful reaction indicates that the serum contained minimal quantities of circulating reagin, not enough to give clear-cut aggregation, yet enough to cause suggestive clumping of the lipoid particles. One of the points of superiority of the precipitation test under discussion is the comparatively small incidence of dubious results (1:140 in the general hospital, 1:65 in the known syphilitic group) as compared with an incidence of 1:70 and 1:13 for the Wassermann reaction. In the total

series of tests, one out of every 26 was Wassermann or Kahn doubtful, while only one in every 107 gave a doubtful precipitation test.

That such doubtful reports cannot be ignored is shown by the fact that of the 241 doubtful precipitation tests in which histories were available for study, 179 were on patients previously diagnosed as syphilitic and treated, 165 of the tests being on patients under active treatment; that 4 more of the 285 presented definite clinical evidence of syphilis; that 13 more were Wassermann positive either at the time of the test or upon subsequent tests; in other words, that at least 212 of the total group were probably syphilitic (Table VI). The incidence of false doubtful reactions (reactions on patients presenting no clinical evidence of syphilis, and in whom all subsequent tests were negative), in the entire series was less than 1-1700 (0.06 per cent); if the calculation is based on the general hospital group alone, it was 1-600. Nevertheless, it must again be emphasized that a single doubtful report per se is not diagnostic or even presumptive evidence of syphilis unless supported by clinical or further serologic proof.

TABLE VI

ANALYSIS OF THE 10,129 POSITIVE AND DOUBTFUL PRECIPITATION REACTIONS IN 26,611 TESTS

	TOTAL NO.	NO. FOR WHICH HISTORIES WERE AVAILABLE	PATIENTS UNDER OBSERVATION OR TREATMENT AS SYPHILITIC	WASSERMANN POSITIVE AT TIME OF PRECIPITATION TEST	CLINICAL EVIDENCE OF SYPHILIS AT TIME OF TEST	WASSERMANN POSITIVE UPON REPEATED TEST	DEFINITE HISTORY OF ANTISYPHILITIC TREATMENT	SYPHILIS PROBABLE FROM HISTORY OF CLINICAL FINDINGS	TOTAL PROBABLY CORRECT TESTS	DATA ON HISTORY INADEQUATE	FALSE REACTIONS	CALCULATED INCIDENCE OF FALSE REACTIONS
Positive Precipitation Test	9844	9476	7627	1372	62	92	235	88	9427	70	28	1:900
Doubtful Precipitation Test	285	241	165	11	4	2	14	16	212	29	11	1:1750
Total	10,129	9,717	7,792	1,383	66	94	249	104	9,639	99	39	1:600

B. *Technical False Positive Reactions.*—In any large laboratory, no matter what the technic used, it is inevitable that there be a certain number of technical false positive reactions, positive results obtained on presumably nonsyphilitic individuals in whom all subsequent tests are negative. The possible causes for false reactions in a precipitation test are numerous and varied: adventitious particles of dirt or denatured protein in the serum mistakenly interpreted as lipoid aggregates; mislabelled specimens; error in the dilution of the antigen and in the amount added, etc. The more delicate the adjustment of the reaction, the more numerous are the technical pitfalls, and the higher the incidence of these errors in technic and interpretation. Conversely, if a given reaction is characterized by a wide margin of safety in the adjustment of the reagents, time of incubation, etc., there are correspondingly fewer technical false positive reactions. Although they may be

readily detected by repeating every positive reaction before instituting treatment, too high an incidnce of such reactions seriously impairs the value of any test.

The incidence of these technical false positive reactions in the general hospital, excluding the group of syphilitic sera, was less than 1:400, that is, 0.25 per cent (Table VII). If this incidence is referred to the total series of 26,641 sera, it becomes less than 1:600. Even this is a maximum figure, inasmuch as many of these cases had only one repeat test and may indeed have been found to be seroposi-

TABLE VII

ANALYSIS OF THE TESTS IN THE GENERAL HOSPITAL AND DISPENSARY POPULATION IN WHICH THE FLOCCULATION REACTION WAS MORE STRONGLY POSITIVE THAN THE WASSERMANN

WASSERMANN	FLOCCULATION	TOTAL NO. OF TESTS	TESTS FOR WHICH HISTORIES WERE AVAILABLE FOR STUDY	PREVIOUSLY DIAGNOSED AS SYPHILIS AND TREATED	CLINICAL EVIDENCE OF SYPHILIS	WASS. LATER POSITIVE	SYPHILIS PROBABLE FROM HISTORY OR CLINICAL FINDINGS	TOTAL PROBABLY CORRECT	DATA ON HISTORY INADEQUATE	FALSE REACTION	CALCULATED INCIDENCE OF FALSE REACTIONS IN THE SERIES OF 16,228 TESTS
−	+	503	411	174	37	50	69	330	54	27	1:425
±	+	161	117	45	15	29	17	106	11	0	0
anticomp.	+	81	47	16	10	13	2	41	5	1	1:800
Total Precipitation positive and Wassermann inconclusive		745	575	235	62	92	88	477	70	28	1:400
−	±	93	63	7	4	1	15	27	27	9	1:700
±	±	13	12	6		1	1	8	2	2	1:6000
anticomp.	±	3	1	1				1			
Total Precipitation doubtful and Wassermann inconclusive		109	76	14	4	2	16	36	29	11	1:600
Grand total		854	651	249	66	94	104	513	99	39	1:250

tive on repeated testing; for it is characteristic of weakly positive sera of syphilitic patients to yield inconsistent, varying results upon repeated tests.

We believe this result compares more than favorably with tests currently used. It is interesting to note that the incidence of technical false positive and doubtful Wassermann reactions was 1:900 and 1:500 respectively, for the general group (Table VIII). To all intents and purposes, therefore, the precipitation test is almost as reliable as the Wassermann test used in our laboratory as regards technical false positive and doubtful reactions.

C. *Biological False Positive Reactions.*—These figures, however, supply only a partial answer to the question of specificity. What interpretation is to be placed upon a persistently positive precipitation test in the absence of clinical signs of syphilis? Does such a series of tests invariably indicate the presence of syphilis; if not, with what frequency is it obtained in nonsyphilitic individuals? For reasons

TABLE VIII

ANALYSIS OF THE TESTS IN THE GENERAL HOSPITAL AND DISPENSARY IN WHICH THE WASSERMANN WAS MORE STRONGLY POSITIVE THAN THE PRECIPITATION TEST

WASSERMANN	PRECIPITATION	TOTAL NO. OF TESTS	TESTS FOR WHICH HISTORIES WERE AVAILABLE FOR STUDY	PREVIOUSLY DIAGNOSED AS SYPHILIS AND TREATED	CLINICAL EVIDENCE OF SYPHILIS	PRECIPITATION TEST LATER POSITIVE	SYPHILIS PROBABLE	TOTAL PROBABLY CORRECT	DATA ON HISTORY INADEQUATE	FALSE	CALCULATED INCIDENCE OF FALSE REACTION IN THE SERIES
+	−	49	32	10	2	5	2	19	2	11	1:900
+	±	11	9			6	1	7	2	0	
Total Wassermann positive and precipitation inconclusive		60	41	10	2	11	3	26	4	11	1:900
±	±	13	12	6		1	1	8	2	2	1:6000
±	−	58	37	5	2	1	4	12	11	14	1:525
Total Wassermann doubtful and precipitation inconclusive		71	49	11	2	2	5	20	13	16	1:500
Grand Total		131	90	21	4	13	8	46	17	27	1:325

which will appear in the following discussion, no absolute answer to these questions is now possible, not only for this reaction, but for any laboratory test for syphilis, including the Wassermann reaction. Based on three distinct lines of evidence, however, the author believes the former statement to be true: *that a repeatedly positive precipitation reaction is as reliable a criterion of syphilitic infection as a repeatedly positive Wassermann reaction; and any patient giving persistently positive tests from a reliable laboratory is to be considered syphilitic, even in the face of a negative Wassermann reaction.*

1. In the first place, among 189 applicants for the blood transfusion donor list, there was only one positive result, the remainder being entirely negative. That one case was found to have a darkfield positive chancre. Only by testing thousands of such subjects, in whom the incidence of syphilis is almost nil, will we arrive at any incontrovertible conclusion as to the absolute specificity of this or any other test for syphilis, including the Wassermann reaction. These 189 cases alone, although suggestive, are too small a series to justify any definite conclusions. We proceed with more indirect and correspondingly less conclusive evidence.

2. Among 946 white patients admitted to the private wards, or observed in a pay diagnostic clinic, patients in whom the incidence of syphilis is lower than in other hospital groups, there were 19 cases with a positive and 7 with a doubtful Wassermann reaction. Of these, 2 and 2 respectively proved to be technical errors, subsequent precipitation test and Wassermann tests being negative. In 4 others (1 positive and 3 doubtful reaction) the test had not been repeated and no deduc-

tion can be drawn from them. There remained for study 17 cases with a persistently positive Wassermann test. The clinical analysis of these cases is given in Table IX. As is there shown, in 4 (23 per cent) of these there was neither history nor clinical evidence of syphilitic infection.

TABLE IX

ANALYSIS OF RESULTS IN 946 WHITE PATIENTS IN THE PRIVATE WARDS OR DISPENSARY; SHOWING THAT A PERSISTENTLY POSITIVE PRECIPITATION TEST, EVEN IN THE FACE OF A NEGATIVE WASSERMANN REACTION, IS AS RELIABLE A CRITERION OF SYPHILIS AS A POSITIVE WASSERMANN REACTION

	TOTAL NO.	TECHNICAL ERROR (POSITIVE ON ONE OCCASION ONLY)	CLINICAL EVIDENCE OF SYPHILIS	DEFINITE HISTORY OF SYPHILITIC INFECTION AND TREATMENT	SYPHILIS PROBABLE FROM HISTORY OR PHYSICAL FINDINGS	NO CLINICAL EVIDENCE; NO HISTORY OF INFECTION OR TREATMENT (LATENT SYPHILIS)
Wassermann positive; precipitation test positive	17	0	8	2	3	4
Wassermann positive; precipitation test negative	2	2	0	0		0
Precipitation test positive; Wassermann negative or doubtful	10	2	2	1	3	2

Accepting the present-day evaluation of a persistently positive Wassermann reaction as pathognomonic of syphilis in a temperate climate, it would thus appear that about one-fourth of all the Wassermann positive cases in this selected series of well-to-do individuals have a *latent* infection, latent even to the point of lacking a history of infection. Every one of these Wassermann-positive cases gave a positive precipitation test. In addition, in the 946 patients there were 8 cases in which the Wassermann was first negative or doubtful, and the precipitation test persistently positive. Two of these presented definite clinical and serologic evidence of syphilis; another was later found to be Wassermann positive; in another case there was a definite history of antisyphilitic intravenous treatment; another later gave a doubtful Wassermann and was clinically and by x-ray findings, probably a case of syphilitic aortitis; another had a hepatitis which cleared under potassium iodide, and had impaired hearing as well; and in only two cases out of 8 was there no evidence or history of syphilis, *approximately the same proportion noted in the Wassermann positive group.*

3. Finally, a clinical analysis of 194 unselected cases in the general hospital and dispensary population in which the precipitation test was persistently positive and the original Wassermann inconclusive, showed that 46 presented clinical evidence of syphilis; 84 others were found to be Wassermann positive upon repeated testing; 20 others gave a definite history of having received antisyphilitic treatment, the majority in our own dispensary; in 7 others syphilis was probable on the basis of the history and clinical findings. In all, 157 cases (81 per cent) presented

more or less definite evidence, either in their history, clinical examination, or subsequent Wassermann tests, of syphilitic infection (Table X).

TABLE X

CLINICAL ANALYSIS OF 194 UNSELECTED CASES IN THE GENERAL DISPENSARY AND HOSPITAL IN WHICH THE PRECIPITATION TEST WAS PERSISTENTLY POSITIVE, AND THE INITIAL WASSERMANN INCONCLUSIVE

WASS.	PRECIPITATION TEST	TOTAL NO.	SYPHILIS PREVIOUSLY DIAGNOSED AND TREATED	CLINICAL EVIDENCE OF SYPHILIS	WASSERMANN SUBSEQUENTLY POSITIVE	SYPHILIS PROBABLE FROM HISTORY OR CLINICAL FINDINGS	NO EVIDENCE OF SYPHILIS (PROBABLY LATENT)
−	+	112	14	24	43	5	26
Anticompl.	+	24		9	13		2
±	+	58	6	13	28	2	9
Total		194	20	46	84	7	37 (19%)

These three lines of evidence are considered adequate proof for the contention that a persistently positive precipitation test, even in a patient whose Wassermann reaction is negative, is as definite evidence for the presence of syphilis as a positive Wassermann reaction.

SUMMARY

The flocculation test devised by the author has proved to be considerably more sensitive than either the Wassermann or Kahn reaction, detecting 75 per cent of 10,383 known syphilitic sera, as compared with 58.7 and 60.2 per cent for the Wassermann and Kahn reaction. It is comparatively free from doubtful results, only one in 137 tests (0.7 per cent) being dubious, as compared with 1.4 per cent for the Wassermann and the Kahn reactions.

In a general dispensary and hospital population (16,228 tests), there were 2117 positive precipitation tests, as compared with 1432 positive Wassermann reactions. In at least 46 untreated cases of clinically active syphilis, the precipitation test was positive, with the Wassermann negative or doubtful.

The incidence of technical false positive and false doubtful reactions in 16,228 tests in a general hospital and dispensary population was 1:400 and 1:600 respectively. In approximately this proportion of cases some error, either of interpretation or technic, led to a positive or doubtful report on a patient presenting no evidence of syphilis, and in whom all subsequent tests were negative. On the other hand, a persistently positive precipitation test was found to be as reliable an indication of syphilis as a positive Wassermann reaction. In 188 selected individuals presumably nonsyphilitic there was not a single positive reaction; in 946 private patients, there were only 8 with a persistently positive precipitation and a negative or doubtful Wassermann. Six of these presented clinical signs, a definite history of syphilitic infection, or a subsequently positive Wassermann reaction. The proportion (2/8) of patients in this group who gave neither history nor clinical signs of syphilis is almost exactly the same as in the Wassermann positive patients of the same group (4/17). Of 194 patients in the general hospital whose precipitation test was persistently positive, though the Wassermann was at first negative or

doubtful, fully 80 per cent gave clinical evidence of syphilis, had a definite history of antisyphilitic treatment, or were later found to have a positive Wassermann reaction. A persistently positive precipitation test, even in a patient whose Wassermann is negative, is therefore believed to be as reliable a criterion of syphilitic infection as a positive Wassermann reaction. In the opinion of the author, it is in itself sufficient evidence for the diagnosis of syphilis.

SOME PROPERTIES OF PNEUMOCHOLIN, A BIOCHEMICAL ANTIGEN*

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THIS paper deals with a new biochemical substance which has been named Pneumocholin. This name has been derived from the first part of the word pneumococcus and from the chemical substance, cholic acid. Pneumocholin itself is a substance of unknown chemical structure produced by the lysis of pneumococci in one of the bile salts. It is probably a protein cleavage product. Some detailed data on the properties and production of pneumocholin are given in this paper. Pneumocholin has been developed as the culmination of more than two years of work and study in an effort to find some specific antipneumococcus agent. This substance has been found to be nontoxic, easily prepared and possessed of marked antigenic properties, producing a high degree of immunity in animals against infection with the pneumococcus.

REVIEW OF THE LITERATURE

It was discovered in 1900 by Neufeld[1] that pneumococci were soluble in bile. It was later found that the bile salts, which are conjugate amino acids (amino-cholic acids), were the constituents of bile responsible for the pneumolytic properties of bile.

From time to time various workers tried the bile salts in a therapeutic manner against lobar pneumonia and other pneumococcus infections.

Barjot[2] treated four men of the black race who were suffering from pneumonia with a 7 per cent solution of sodium taurocholate, containing 2 per cent of magnesium sulphate, intravenously. He reported his results in 1928.

Another investigator in this field was A. Castellanos y Gonzalez[3] who reported his work in several papers during 1929. This worker treated three children suffering from empyema by injecting bile salts intrapleurally. He also treated a patient having meningitis and one suffering from Pneumococcus septicemia. He reported his results as being very good.

Early in 1930 the author treated three cases of typical pneumococcus lobar pneumonia with bile salts intravenously.[4] This work brought out the fact that although the bile salts were effective in rapidly terminating the pneumococcus infection, their local toxic action on the veins at the site of injection was too great.

Work was then directed towards finding a derivative of the bile salts which would have a maximum pneumolytic power and a minimum toxic action on animal tissue. This led to the investigation of sodium dehydrocholate which was the subject of a paper published in June, 1931.[5] It was found that sodium dehydrocholate dissolved pneumococci when they were taken directly from the animal body but failed in dissolving pneumococci that were grown in artificial culture media. Sodium dehydrocholate has not yet been tried in clinical work.

*Received for publication, June 29, 1932.

Early in 1931 work was done with sodium dehydrocholate to determine its effect on pneumococci and on tissues. The results of this work have been reported.[6] It was discovered in this work that pneumococci when treated with sodium dehydrocholate stimulated a degree of immunity when injected into rabbits. These chemically treated pneumococci were found to possess antigenic properties which other types of pneumococcus vaccines do not have. Adequate clinical trial of this particular bile salt derivative, sodium dehydrocholate, in the treatment of pneumococcus infections has not been made.

In 1930, Ross published an important paper.[7] In this he described the use of bile salt dissolved pneumococci to produce a high degree of immunity in rats. He fed the bile salt dissolved pneumococci to the rats in their food. Ross also used macerated and hydrochloric acid treated pneumococci in his work, but these became bile treated when they reached the animals' duodenum. These latter two preparations of pneumococci were also reported to have given somewhat less effective results than the pneumococci treated with the bile salts initially.

Elton by his work[8] has shown that patients with lobar pneumonia have increased icterus indices and positive Van den Bergh tests. His work shows that bile constituents are increased in the blood of such cases, which supports the theory that bile salts are important in the recovery mechanism.

EXPERIMENTAL WORK

The work of this paper deals with the production of pneumocholin, the effect of pneumocholin in the animal body, and other properties of pneumocholin.

PRODUCTION OF PNEUMOCHOLIN

Many obstacles had to be overcome in the production of pneumocholin. Large amounts of pneumococci are needed and the pneumococcus does not grow very luxuriantly in artificial media.

The propagation of the pneumococci was effected by growing them on blood agar. The plates were always heavily inoculated.

Pneumococci used in the production of pneumocholin were transferred from the blood agar and grown in a type of hormone broth. This broth was made as follows:

Meat extract	3 gm.
Peptone	20 gm.
Sodium chloride	5 gm.
Distilled water	1000 c.c.

These ingredients are placed in a flask, then two pieces (about two cubic inches) of fat-free beef heart or veal are added. The flask and ingredients are now placed in the autoclave for fifteen minutes at 15 pounds' pressure. Adding the meat in this manner lessens the amount of precipitate that is formed in the media. After removing the flask from the autoclave the media is adjusted to a P_H of 8.4 and allowed to stand overnight. The broth is now decanted and poured in smaller flasks or tubed. A chip of marble is placed in each tube or flask and the broth is again autoclaved at 15 pounds' pressure for twenty minutes. The standard hormone broth is not very satisfactory as it usually contains more precipitate, but it grows the organisms well. Ascitic fluid broth and serum broth are excellent for growing the pneumococci.

The broth is now inoculated with pneumococci. This is done by cutting out a square of media with a platinum wire loop from a blood agar plate containing pneumococcus colonies and placing this piece of media in the broth.

Maximum pneumococcus growth is usually obtained in forty-eight hours at 37° C.

This pneumococcus broth culture is now filtered through a sterile, coarse paper filter into a sterile flask. This removes any solid particles or precipitate from the broth and allows most of the organisms to pass through. The pneumococcus broth culture is now placed in centrifuge tubes and centrifuged at high speed until the broth becomes clear and the organisms are precipitated in the bottom of the tube. The broth is now poured out of the tubes or drawn out with a vacuum pump. The centrifuge tubes are refilled with the pneumococcus broth culture and the process repeated until enough pneumococci are obtained, packed in the bottom of the tubes. The centrifuge tubes and contents are kept sterile with caps. Paper caps were used in this work.

When the last portion of broth has been removed from the centrifuge tubes, the pneumococci are resuspended in 0.85 per cent sodium chloride solution containing 2 per cent of sodium taurocholate.* The centrifuge tubes are now placed in the incubator for twenty-four hours. This product is pneumocholin and is ready for intravenous injection. It is well to seal the pneumocholin in ampules for later use.

In the above work 30 c.c. of a solution of sodium taurocholate 2 per cent and sodium chloride 0.85 per cent was added to each 0.1 c.c. of packed organisms.

The bile salt, sodium taurocholate was selected for this work because it is the least toxic of the natural bile salts, is effective in producing lysis and is readily available commercially. Many of the cholic acid salts or conjugates would be available to produce pneumocholin.

Pneumocholin itself has a certain solubility and if made too concentrated will produce a cloudy suspension and gradually precipitate. This usually happened when the above product was placed in the refrigerator. On mild heating, solution would again take place. Further purification of pneumocholin can undoubtedly be effected by taking advantage of its relative insolubility. Further quantitative work and other chemical experiments will have to be performed in order to standardize pneumocholin.

ANTIGENIC EFFECT OF PNEUMOCHOLIN IN THE ANIMAL BODY

Due to the amount of work involved, experiments thus far have been confined to Type I pneumococci.

Virulence was maintained by recovering the pneumococci from the control rabbits which died, or by passing the organisms through mice.

Virulent pneumococci as used in the experiments were recovered from an animal dying from a pneumococcus infection. The organisms were passed from the animal to a blood agar plate, to broth. No more artificial growth than this was allowed. The broth was then used to inject the experimental animals. Incubation in every case was for twenty-four hours. In inoculating broth, it was found effective and reliable to cut a small square from the blood agar plate with a platinum wire loop and to transfer this block of media to the flask of

*The sodium taurocholate used in this work was obtained from the Difco Laboratories, Detroit, Mich.

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*The sodium taurocholate used in this work was obtained from the Difco Laboratories, Detroit, Mich.

broth. If only small amounts of pneumococci are transferred, sometimes growth will not occur. For this reason the above method is used.

The organisms used in this work were obtained in pure culture.* When the organisms were received they were passed through mice to enhance their virulence. The identity of the organism was checked culturally, serologically and tested for bile solubility.

Rabbits which died were autopsied in each experiment and the pneumococci recovered by streaking on blood agar plates.

Care was taken to have but one variable factor in each experiment.

Injections were made intravenously. The pneumocholin was made in the manner described above.

The rabbits used in the following experiments were of fairly uniform size, ranging from 2 to 3 kg. in weight. Therefore the factor of weight was ignored.

EXPERIMENT I (TABLE I)

Effect of Pneumocholin (Five Minute Experiment).—This experiment was performed to see if pneumocholin would save rabbits from pneumococci when the two were administered intravenously at about the same time. As the experiment shows, the pneumocholin did not protect these rabbits.

On the hypothesis that a certain length of time was necessary for the pneumocholin to stimulate the production of immunity the following experiments were undertaken.

TABLE I

EFFECT OF PNEUMOCHOLIN (FIVE MINUTE EXPERIMENT)

RABBIT	FEBRUARY 3, 1932 PNEUMOCHOLIN		PNEUMOCOCCI*	RESULT DAYS
1 C	....		0.1 c.c.	D 1
2	0.5 c.c.	Interval	0.1 c.c.	D 1
3	0.5 c.c.	5 Min.	0.1 c.c.	D 1
4	0.5 c.c.		0.1 c.c.	D 2

*Twenty-four-hour broth culture, Type I. Injections made intravenously.
C = control; D = died.

EXPERIMENT II (TABLE II)

Effect of Pneumocholin (Seven and Nine Day Experiment).—It was found that pneumocholin produced an immunity to large fatal doses of pneumococci when they were injected seven and nine days following the pneumocholin. The test rabbits in this experiment were not made sick by either the pneumocholin or the fatal doses of organisms. The control rabbit died in two days.

The pneumocholin used in this experiment was seven days old. It was kept in the refrigerator in a cotton stoppered flask.

EXPERIMENT III (TABLE III)

Lack of Immunity in One or Two Days.—When Experiment II revealed the fact that pneumocholin produced a marked immunity against pneumococci, it was thought desirable to determine as accurately as possible just when the immunity appeared. To this end Experiment III was performed to see if any immunity developed in one or two days. This experiment proved that no

*The pneumococci were obtained from the Mulford Laboratories of Sharp & Dohme, Philadelphia, Pa.

demonstrable immunity was developed in this length of time. Experiment IV which follows, is considered the most important experiment in this work. It shows very definitely when the immunity induced by pneumocholin appears.

TABLE II

EFFECT OF PNEUMOCHOLIN (SEVEN AND NINE DAY EXPERIMENT)

RABBIT	FEBRUARY 9, 1932 PNEUMOCHOLIN	INTERVAL DAYS	PNEUMOCOCCI*	RESULT DAYS
5	1 c.c.	7	0.2 c.c.	S
6	2 c.c.	7	0.2 c.c.	S
7	1 c.c.	9	0.4 c.c.	S
8	2 c.c.	9	0.2 c.c.	S
9 C	....	..	0.2 c.c.	D 2

Pneumocholin used was made on February 2, 1932.
*Twenty-four-hour broth culture of pneumococci given intravenously.
S= survived; C = control; D = died.

TABLE III

LACK OF IMMUNITY IN ONE OR TWO DAYS

RABBIT	FEBRUARY 22, 1932 PNEUMOCHOLIN	INTERVAL DAYS	PNEUMOCOCCI*	RESULT DAYS
10	2 c.c.	1	0.2 c.c.	D 1
11	2 c.c.	1	0.4 c.c.	D 1
12	5 c.c.	1	0.4 c.c.	D 1
13	5 c.c.	2	0.2 c.c.	D 1
14	5 c.c.	2	0.4 c.c.	D 3
15	4 c.c.	2	0.4 c.c.	D 3
16 C	....	..	0.2 c.c.	D 2

*Twenty-four-hour broth culture pneumococci Type I, injected intravenously.
C = control; D = died. Pneumocholin made on February 2, 1932.

EXPERIMENT IV (TABLE IV)

Immunity Appears Between Three and Four Days.—This experiment was devised in order to determine when immunity appears following an injection of pneumocholin. Injections were made intravenously. After giving pneumocholin, intervals of from three to seven days were allowed to elapse, and on each of these successive days a group of rabbits including a control, were given fatal doses of organisms.

The experiment showed that immunity began to appear on the third day and on the fourth day was great enough to save all the test animals.

The organisms used were very virulent and were given in doses designed to kill the controls in a short time. This was necessary to make the results of the experiment clear cut.

A series of experiments done at different times have been collected here as Experiment IV. In this paper there are nine experiments which are clear cut and show definitely that pneumocholin produces a marked immunity to the pneumococcus. These experiments confirm each other in every instance where four days or more elapse after injecting pneumocholin.

EXPERIMENT V (TABLE V)

Immunity Present After 49 Days.—This experiment was performed to determine if the immunity to pneumococci, induced by the pneumocholin, lasted for an appreciable length of time. The experiment shows conclusively that the immunity which these rabbits acquired in Experiment II was still present forty-nine days later. Since the immunity was so definite after this

TABLE IV

IMMUNITY APPEARS BETWEEN THREE AND FOUR DAYS

RABBIT	PNEUMOCHOLIN	INTERVAL DAYS	PNEUMOCOCCI*	RESULT DAYS
17	2 c.c.	3	0.3 c.c.	S
18	2 c.c.	3	0.3 c.c.	D 2
19 C	....	..	0.3 c.c.	D 1
20	2 c.c.	4	0.3 c.c.	S
21	2 c.c.	4	0.3 c.c.	S
22	2 c.c.	4	0.3 c.c.	S
23 C	....	..	0.3 c.c.	D 1
24	2 c.c.	5	0.2 c.c.	S
25	2 c.c.	5	0.2 c.c.	S
26 C	....	..	0.2 c.c.	D 1
27	2 c.c.	6	1 c.c.	S
28	2 c.c.	6	1 c.c.	S
29	2 c.c.	6	1 c.c.	S
30 C	....	..	1 c.c.	D 4
31	2 c.c.	7	0.3 c.c.	S
32	2 c.c.	7	0.3 c.c.	S
33	2 c.c.	7	0.3 c.c.	S
34 C	....	..	0.3 c.c.	D 2

*Twenty-four-hour broth culture, Type I, injected intravenously.
C = control; D = died; S = survived.

TABLE V

IMMUNITY PRESENT AFTER FORTY-NINE DAYS

RABBIT	FEBRUARY 9, 1932 PNEUMOCHOLIN	INTERVAL DAYS	MARCH 30, 1932 PNEUMOCOCCI*	RESULT DAYS
5	1 c.c.	49	1 c.c.	S
6	2 c.c.	49	1 c.c.	S
7	1 c.c.	49	1 c.c.	S
8	2 c.c.	49	1 c.c.	S
35 C	....	..	1 c.c.	D 2
36 C	....	..	1 c.c.	D 4

*Twenty-four-hour broth culture, Type I, injected intravenously.
C = control; S = survived; D = died.

length of time it probably lasts much longer. How long it will last will remain for future experiments to demonstrate.

EXPERIMENT VI (TABLE VI)

Pneumocholin Tried Against Septicemia.—In this experiment the organisms used were grown on blood agar plates for four times after recovering them from a control rabbit which died, and then grown for twenty-four hours in broth. Pneumocholin was given intravenously one day following the organisms. The control rabbit died in seven days and the test rabbits survived although they became sick. These test rabbits did not eat well for several days and lost weight. They eventually recovered and regained their weight. The test rabbits were saved from a fatal termination which would have been produced by a pneumococcus septicemia. It is of course not known yet whether pneumocholin could have any effect on the course of a lobar pneumonia already established.

This experiment suggests that pneumocholin might be useful in a therapeutic way because of the short time in which immunity is developed. Vaccines of dead or avirulent pneumococci do not produce any practical degree of immunity but it appears that the broken down pneumo-

cocci induce immunity in a short time. Although whole pneumococci do not act as effective antigens, bile salt dissolved pneumococci do. It can only be speculated as to what occurs when pneumococci are dissolved in bile salts. Probably protein cleavage products of some kind are formed. It will remain for advancing organic chemistry to fully explain pneumocholin.

TABLE VI

PNEUMOCHOLIN TRIED AGAINST SEPTICEMIA

RABBIT	FEBRUARY 23, 1932 PNEUMOCOCCI*	FEBRUARY 24, 1932 PNEUMOCHOLIN	RESULT DAYS
37 C	0.4 c.c.	....	D 7
38	0.4 c.c.	2 c.c.	S
39	0.4 c.c.	2 c.c.	S
40	0.4 c.c.	2 c.c.	S

*Twenty-four-hour broth culture, Type I, attenuated; given intravenously. Pneumocholin made on February 2, 1932.
C = control; S = survived; D = died.

EXPERIMENT VII (TABLE VII)

No Immunity Produced by Avirulent Organisms.—In this experiment, test rabbits were given several intravenous injections of avirulent but living pneumococci at intervals of five to seven days. In the case of two rabbits, Rabbits 41 and 43, four injections of avirulent organisms were given. In the case of Rabbit 42, only two injections of avirulent organisms were given. Seven days after the last injection of avirulent pneumococci the test rabbits and a control were given doses of virulent pneumococci. The results are shown in Table VII. The avirulent pneumococci did not produce any appreciable immunity in the test rabbits.

It appears that whole pneumococci do not have the antigenic properties that bile salt dissolved pneumococci have. This of course is not new and merely confirms previous findings and general clinical experience with pneumococcus vaccines.

A theory advanced here is that the bile salts may dissociate the pneumococcus protein from the pneumococcus carbohydrate and thus allow the free pneumococcus protein to act as an antigen.

TABLE VII

NO IMMUNITY PRODUCED BY AVIRULENT ORGANISMS

RABBIT	AVIRULENT PNEUMOCOCCI*				PNEUMOCOCCI** MARCH 30, 1932	RESULT DAYS
	MARCH 4, 1932	MARCH 9, 1932	MARCH 16, 1932	MARCH 23, 1932		
41	0.2 c.c.	0.3 c.c.	0.4 c.c.	1 c.c.	1 c.c.	D 2
42	....	....	0.4 c.c.	1 c.c.	1 c.c.	D 4
43	0.2 c.c.	0.3 c.c.	0.4 c.c.	1 c.c.	1 c.c.	D 2
44 C	....	....	....	....	1 c.c.	D 2

*Twenty-four-hour broth culture, avirulent strain.
**Twenty-four-hour broth culture, Type I, virulent strain.
C = control; D = died.

EXPERIMENT VIII (TABLE VIII)

Effect of Heated Pneumocholin.—This experiment was performed to see if pneumocholin was heat stable (at 56° C. for thirty minutes). The results show that pneumocholin is stable to this mild degree of heat. As this degree of heat kills most vegetative forms of pathogenic organisms, the method is available to safeguard the sterility of pneumocholin. The pneumocholin used in this and the previous experiments was made on February 2, 1932 showing that this substance remains stable for a period of time, over three months so far.

TABLE VIII

EFFECT OF HEATED PNEUMOCHOLIN

RABBIT	MAY 14, 1932 PNEUMOCHOLIN*	MAY 19, 1932 PNEUMOCOCCI**	RESULT DAYS
5 C	....	0.3 c.c.	D 2
34	2 c.c.	0.3 c.c.	S
54	2 c.c.	0.3 c.c.	S
56	2 c.c.	0.3 c.c.	S

*Pneumocholin was heated at 56° C. for thirty minutes.
**Twenty-four-hour broth culture, Type I, given intravenously.
C = control; S = survived; D = died.

EXPERIMENT IX

Intravenous Injections in Man.—Pneumocholin as made above was filtered through a Berkefeld -N filter, sealed in sterile ampules and then subjected to heating at 56° C. for thirty minutes.

This product, doubly protected against contamination was cultured on various media including blood agar and was found to be sterile.

Eight cubic centimeters of pneumocholin prepared and checked as above were administered intravenously to each of ten people. There were no harmful effects whatever, either locally or generally.

DISCUSSION

Whole pneumococci lack adequate antigenic properties. This is demonstrated by an experiment in this paper and in general by the experience of the medical profession with pneumococcus vaccines.

The pneumococcus when dissolved with a minimum and negligible amount of bile salt forms a substance having marked antigenic properties. This substance, called pneumocholin, is nontoxic either locally or generally when injected intravenously. Subcutaneous injection is not advised.

Although pneumocholin was made in this work with pneumococci and sodium taurocholate, the idea of using sodium dehydrocholate as in former work[6] has not been entirely abandoned. From a certain viewpoint, however, it is probably best to use pneumococci which are completely dissolved when preparing pneumocholin for intravenous injection. The soluble type of pneumocholin as made with sodium taurocholate is also filterable. Filtration can be used as an extra safeguard to insure sterility.

Sterility of pneumocholin can be maintained by observing aseptic technic in its preparation and also by pasteurization.

It has been found that pneumocholin is stable for a period of months when kept in the ice box in cotton stoppered flasks. Sealed ampules of pneumocholin are being kept for experiments at a later date. Some of these ampules are kept at room temperature and some in the refrigerator.

The experiments show that when pneumocholin is injected intravenously, immunity appears between the third and fourth day.

Work with Types II, III and Group IV pneumococci will be made the subject of a future paper.

As the bile salts dissolve all types of pneumococci it is believed that an effective pneumocholin can be prepared for each type. It is further believed that pneumocholin is not type specific. This however remains to be demonstrated.

There are three potential uses of pneumocholin. The first and most important use would be for prophylaxis. The extent to which pneumocholin might be useful as a prophylactic agent will depend upon the length of time that immunity is conferred. How long the immunity will last can only be determined by future experiments and experience. If immunity were to last only for three or four months it would be worth while to use pneumocholin, especially in such places as military camps and during epidemics. Doctors, nurses, and others exposed to pneumonia patients could well afford to use such a prophylactic agent. If immunity conferred by pneumocholin lasts for a longer time, it will be a correspondingly more valuable agent.

A second possible use would be for therapy. It may be that pneumocholin can be used in the treatment of pneumococcus infections to increase the body's resistance. The experiments indicate that fatal terminations could not be intercepted unless there were a grace period of three to four days, after treatment with pneumocholin. Therapeutic experiments will of course be necessary to prove or disprove this theory.

A third use would be for antiserum production. This, so far, is entirely an hypothetical proposition. It is possible that pneumocholin will be found useful in the production of antisera when injected into animals, in a manner analogous to the production of diphtheria or scarlet fever antisera.

CONCLUSIONS

1. Pneumocholin is a purefied product, produced by solution of pneumococci in a bile salt.

2. Pneumocholin has remained stable in time, under conditions of refrigeration. The maximum time in which it will be stable has not yet been determined.

3. Pneumocholin has remained stable to moderate heat, 56° C. for one-half hour (pasteurization).

4. Pneumocholin, when injected intravenously in rabbits, induces a very effective immunity to pneumococci after three or four days. Only pneumocholin made from Type I pneumococci has been investigated.

5. Pneumocholin promises to be a valuable prophylactic agent against pneumococcus infections and produces no deleterious effects when injected intravenously.

NOTE.—The work of this paper was performed in the laboratory of the Veterans Administration Hospital, Coatesville, Pa. It was done after working hours and at the author's expense.

I wish to thank Dr. Philip B. Matz and Dr. A. H. Pierce for permitting this work to be done in the above hospital and for their words of encouragement.

REFERENCES

1. Neufeld: Ztschr. f. Hyg. u. Infektionskrankh. **34**: 454, 1900; Arb. a. d. k. Gsndhtsamte. **25**: 494, 1907.
2. Barjot: The Treatment of Pneumococcus Septicemia by the Intravenous Injection of Bile Salts, Bull. Acad. de med., Paris **100**: 898, 1928.
3. Castellanos y Gonzales, A.: Rev. de med. y ciruj. de la Habana **34**: 133, 1929.

4. Ziegler, E. E.: The Specific Effect of Bile Salts on Pneumococci and on Pneumococcus Pneumonia, Arch. Int. Med. **46**: 644-656, 1930.
5. Ziegler, E. E.: Sodium Dehydrocholate, Its Specific Effect on Pneumococci, II, J. Lab. & Clin. Med. **16**: 868, 1931.
6. Ziegler, E. E.: The Effects on Pneumococci of Sodium Dehydrocholate, A Bile Salt Derivative, III, J. Lab. & Clin. Med. **17**: 317, 1932.
7. Ross, V.: Oral Immunization Against the Pneumococcus; Use of Bile Salts Dissolved Organisms, etc., J. Exper. Med. **51**: 585, 1930.
8. Elton, N. W.: Latent Jaundice of Lobar Pneumonia. J. Michigan M. Soc. **28**: 451, 1929; Icterus Index Studies in Lobar Pneumonia, II, New England J. Med. **201**: 611, 1929; Serum Pigmentation and Kinetics of Latent Jaundice of Lobar Pneumonia, J. Detroit Coll. Med. & Surg. **1**: 34, 1930.

THERAPEUTIC USE OF IODIZED OIL IN PULMONARY DISEASE*

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SINCE the report of Sicard and Forestier,[1] in 1922, relative to the use of iodized oil in the tracheobronchial tree, the utilization of this radiopaque substance has become quite general as a diagnostic aid, and current literature is replete with references to its usage. The remedial possibilities from its employment have also been referred to by several authors, and results reported by a few, but the larger percentage of physicians are unaware of the therapeutic value of this agent. During the past five years we have used iodized oil therapy in more than one hundred and fifty patients with pulmonary disease. In 1927 we began giving the oil therapeutic trial in selected patients with bronchitis and bronchiectasis where all other measures had failed. Encouraged by results we broadened its usage until we now unhesitatingly use iodized oil therapeutically in almost any patient suffering with nontuberculous lung disease, and even in selected tuberculous patients. Although Pritchard, Whyte and Gordon,[2] Ochsner,[3] and others had previously reported good therapeutic results, we were not sufficiently impressed with the possibilities of this procedure until convinced, by improvement observed in patients in whom the oil had been used diagnostically.

In our early experience with iodized oil we observed an occasional reaction.[4] Two patients developed symptoms of mild iodism, which we attributed to the presence of oil in the stomach.[2] An occasional patient complained of symptoms such as discomfort in the chest and increased cough, probably caused by a deterioration due to age, giving the oil a dark color; possibly with liberation of free iodine. This also was reported by Pritchard, Whyte and Gordon,[2] in April, 1926, but was not sufficiently appreciated by us until we encountered similar reactions. Since we have learned to avoid allowing oil to enter the stomach and no longer use any oil that has turned brown, we have no such reactions. A reaction similar to that encountered in vaccine therapy, with symptoms of aching and chilliness, followed by temporary fever, is occasionally reported by bronchiectatic patients after the first introduction of oil. We have observed this phenomenon chiefly among patients

*Received for publication, July 3, 1932.

showing some evidence of atelectasis, and have also noted that many of these derive the greatest benefit from the treatment.

KIND OF OIL USED

Any of the standard iodized oils may be employed, but we now use lipoiodine exclusively, because it is comparatively thin, quite volatile, easy to administer, and free from irritant action. Most patients prefer this oil as it can be taken with but little discomfort. If the oil is heated to 101 to 102° F., it is given more easily and is more soothing to the average patient. Due to the warmth, patients can feel the oil pass through the bronchial tree and many say this is quite soothing. This is especially true of patients having acute laryngitis, tracheitis, asthma, and asthmatic bronchitis.

METHOD OF GIVING IODIZED OIL

Various methods for introducing oil into the tracheobronchial tree have been described, but we prefer the supraglottic method as follows:

The patient is seated facing the operator, inclined towards the side into which it is desired to introduce the oil. He is then instructed to relax and breathe quietly, allowing his tongue to rest comfortably on the floor of the mouth. The pharynx, larynx and upper portion of the trachea are then sprayed with a small quantity of 1 per cent cocaine solution and the warm oil (101 to 102° F.), is dropped directly into the trachea from a 10 c.c. syringe with attached laryngeal cannula. By using a laryngeal mirror the oil can be so dropped into the trachea that flooding of the epiglottis and larynx is eliminated, and the possibility of oil being swallowed is avoided. This is possible without the use of a mirror, but in our experience, less satisfactory.

AMOUNT OF OIL USED

When doubtful as to the patient's tolerance for oil or iodine, and in acute laryngitis and tracheitis, as little as 5 to 10 c.c. of lipoiodine have been given initially, but usually 10 to 20 c.c. are administered at the first treatment. Daily progress notes setting forth symptoms and any reaction following each treatment are made. Oil is given daily for two or three or more treatments, or less frequently, depending on symptoms, fluoroscopic appearances and when necessary, x-ray studies. Effort is made to record so far as possible appearances in the trachobronchial tree, to determine the underlying pathology, the rapidity of absorption or elimination of the oil and the amount of oil remaining or accumulating in the tracheobronchial tree. After the first treatment or course of treatment the patient is advised to return if and when further treatment is desired. Usually within two or three weeks the patient will return for further treatment, although occasionally symptoms are relieved for six to twelve months.

ELIMINATION OF OIL

We find that the oil is eliminated rapidly in some patients and very slowly in others. In two patients with similar conditions, treated alike, the oil may disappear in one after a few days and be observed in the bronchial tree of the other after four to six months. It is infrequently expectorated after the first day or two and occasionally a patient reports tasting the oil after periods of weeks or months.

CONTRAINDICATIONS

Pneumonia, acute lung abscess and acute exudative tuberculosis are believed contraindications for the use of iodized oil, but since we have not tried it in either of these conditions we are unable to speak from experience. Because it is of such proved value in atelectasis of the lung we think it may be valuable in certain pneumonias of atelectatic origin.

Resultant iodism, sepsis, laryngeal edema, drowned lung, and spreading of infection from diseased to healthy lung have frequently been mentioned as dangers,[5] but our experience does not confirm this. We believe that with proper care there is but little risk from any of these.

Acute laryngitis, tracheitis, and bronchitis have been mentioned as contraindications, but again our experience does not support this. On the contrary, it is in this group of patients that we believe oil probably has its greatest field of usefulness.

FUNGUS DISEASE AND VINCENT'S INFECTION

Seven patients with fungus disease of the lung were given iodized oil. Of these there were five with Moniliasis, one Streptothrix and one Aspergillosis. Of these four were markedly improved with complete symptomatic relief and one was apparently cured. In one there was improvement, but not entire relief of symptoms. In one there was no improvement.

Three patients with Vincent's infection were treated with iodized oil, two were cured. One patient with advanced gangrene of the lung improved temporarily after the introduction of oil, but ultimately died following surgical intervention.

	NO. CASES	MARKEDLY IMPROVED	IMPROVED	UNIMPROVED	MADE WORSE
Atelectasis	5	5	0	0	0
Asthmatic bronchitis Bronchial asthma	26	14	12	0	0
Bronchiectasis	30	20	8	2	0
Chronic bronchitis	44	20	15	9	0
Chronic lung abscess	4	0	4	0	0
Chronic empyema	3	1	2	0	0
Acute respiratory infection (laryngitis, tracheitis, bronchitis)	20	15	5	0	0
Moniliasis	5	5	0	0	0
Streptothrix	1	1	0	0	0
Vincent's Infection	3	2	0	1	0
Aspergillosis	1	1	0	0	0

CHRONIC LUNG ABSCESS AND EMPYEMA

Iodized oil was used therapeutically in four patients with chronic lung abscess, and three patients with empyema. All were symptomatically improved. Two of the empyemas were tuberculous and were cured by aspiration and irrigation, followed by intrapleural instillation of lipoiodine and tincture of gentian violet. Except to state that the oil did none of them the slightest harm we can draw no conclusions from so small a number.

BRONCHIECTASIS

Thirty patients with severe bronchiectasis were treated with iodized oil. Twenty were markedly benefited, eight slightly to moderately improved, and two showed no improvement. None were made worse.

CHRONIC BRONCHITIS

Forty-four patients with chronic bronchitis were treated with iodized oil. Thirty-five were improved, nine unimproved.

ATELECTASIS OF THE LUNG

Five patients with atelectasis were treated with iodized oil, all of whom were very definitely benefited. Usually in a few hours after the introduction of oil, the patients cough and expectorate copiously, and practically all state that they immediately feel better. On the following day some of these patients are symptom free.

BRONCHIAL ASTHMA

With great caution last year we began using iodized oil therapeutically as a measure of last resort in patients having asthmatic bronchitis with severe recurrent bronchial asthma, where practically all other known remedies had failed or ceased to afford relief. Improvement in some was so prompt and gratifying that we were encouraged to give this treatment further trial and have used it in twenty-six patients. With no patient has there been failure to give some relief. At first we feared the introduction of oil might precipitate an attack of asthma, but in no instance has this happened. Only once has it been necessary to use adrenalin, which is kept constantly available. On the contrary, in many instances the asthma has promptly subsided following introduction of oil. We feel that asthma which is primarily allergic should be treated for this before trying oil, but if and when these measures fail or cease to afford relief, iodized oil should be administered. Many of these patients have emphysema and patchy atelectasis with a complicating lung infection which may be the explanation for the response to iodized oil therapy. We have not used oil long enough or on a sufficient number of asthmatics to know in whom or what type of condition to exclude its use, but commend its trial in all asthmatics not responding to other forms of therapy. It would probably be a safe precaution to do a preliminary skin test for sensitiveness.

ACUTE LARYNGITIS AND TRACHEITIS

Twenty patients with acute laryngitis and tracheitis have been treated with iodized oil and all experienced relief. In ten the relief of symptoms was immediate; five were relieved within one to two hours; and five other patients experienced some relief, but subsidence of symptoms was slow and gradual. The marked relief of symptoms in these patients has been surprising and gratifying, but the number is too few on which to base any conclusions, however, in no instance has the treatment done the slightest harm.

ILLUSTRATIVE CASES

Spirochetosis.—Mr. E. B., an attorney, aged thirty-nine, first consulted us in August, 1930, complaining of chronic cough, expectoration and recent hemoptysis.

Family history was negative. He had had pneumonia during childhood, typhoid fever in 1907, influenza and rheumatism while in France during the war with a recurrence in 1926. A history of morning cough and expectoration dated back for several years. He had noticed that smoking seemed to irritate his throat, causing an inclination to clear the throat with a slight hacking cough, which had become a habit. Hemoptysis two days previously brought the patient for examination. Physical examination gave evidence of an atypical lesion of the left lung with a few small and medium moist râles throughout the lower half. X-ray of chest showed diffuse mottling throughout the left lung which was atypical, but suggestive of an early miliary tuberculosis or a fungoid infection. Repeated sputum studies were negative for tubercle bacilli, but Friedlander's bacillus and numerous spirochetal organisms were cultured from the sputum.

The patient was given massive doses of potassium iodide and small doses of arsphenamine and autogenous vaccine, with no improvement at the end of three weeks. We then decided to try iodized oil, introducing 20 c.c. into the tracheobronchial tree. Within forty-eight hours, cough and expectoration were relieved and all toxic symptoms disappeared. The patient continued symptom free for four weeks. With return of cough and fatigue he was again treated with the same result. Interval treatments were continued over a period of six months, when he was discharged as apparently cured. He has remained well.

Chronic Bronchitis.—Miss A. R., an executive, aged fifty-two, first consulted us in June, 1928, because of a spasmodic nonproductive cough occurring and recurring inopportunely, thus causing her untold embarrassment. Her mother had died of tuberculosis and patient had had pneumonia three times, otherwise family and past history were irrelevant. Present symptoms were of nine months' duration; onset with an acute respiratory infection complicated by empyema of the left antrum, which had been adequately treated. Physical examination showed small moist râles scattered throughout the bases of both lungs posteriorly, otherwise negative. X-ray of chest showed marked thickening of the bronchi to both bases, otherwise both physical examination and laboratory studies were essentially negative. The patient was given 10 c.c. of iodized oil into the base of each lung. Fluoroscopic observation and x-ray studies showed the oil well distributed in the lung bases. There was evidence of multiple bronchial dilatations, but no bronchiectatic pockets. Patient stated that she received relief from her desire to cough. She was instructed to return whenever she felt the need of more oil, so reappeared in two months stating that she had been relieved completely of her symptoms, but was again becoming annoyed by a desire to cough. She was again given iodized oil and continued to receive these treatments at approximately two month intervals. Meanwhile she remained entirely free from cough, stating that she usually began to feel the need of oil at the end of seven or eight weeks. She remained well and active with no other treatment until the fall of 1930, when she died following an abdominal operation.

Bronchial Asthma.—Mr. F. O. B., a chemist, aged fifty, first consulted us on Feb. 1, 1932, complaining of asthma. Illness began following influenza in 1918 as spasmodic attacks of cough and expectoration at infrequent intervals. In 1923 he had had a mild left sided pleurisy and in 1928 he had a recurring bronchitis. In

1930 he experienced chest pain that was diagnosed intercostal neuralgia. In 1931 he had an automobile accident with fracture of two ribs followed by the first development of definite asthma. Thorough study was made at an excellent asthma clinic, but no etiologic cause could be found, except pansinusitis and diseased tonsils. The sinuses were adequately treated and tonsillectomy performed, but there was no improvement in the asthma. When seen by us he had been unable to eat or sleep and was extremely nervous, due to severe asthma for the preceding ten days. Physical examination showed moderate emphysema with sibilant and sonorous râles throughout the chest. Five cubic centimeters of iodized oil were introduced into each lung. Patient was given ephedrin with a sedative. Two days later he returned somewhat improved but still having asthma. Three days later the patient was admitted to hospital and 10 c.c. of iodized oil introduced into each lung for three successive days. Asthma was promptly relieved and he has remained comfortable since that time. It has been necessary to administer oil at intervals of five to six weeks. Recently he developed an upper respiratory infection precipitated by exposure and complicated by empyema of the antrum. He was given another iodized oil treatment which relieved the symptoms of laryngitis and tracheitis. Patient has had no further trouble.

Bronchiectasis.—Miss E. E., a student, aged sixteen, first consulted us in April, 1929, complaining of cough and expectoration. Family history was irrelevant. Present symptoms developed following whooping cough when the patient was three years old. At the age of four years she had developed an empyema of the right antrum which was drained. Following this illness she had repeated attacks of bronchitis and pneumonia which confined her to bed several times each year. During the last four years the patient had had hypothyroidism with an average metabolic reading of –24, requiring nine grains of thyroid extract daily to maintain a metabolic balance. Patient appeared well nourished, but anemic. Examination showed bronchial râles throughout both lungs with showers of small and medium moist râles at both bases. X-ray of chest showed thickening at both bases, suggestive of bronchiectasis. Examination of nose showed heavy mucopurulent discharge into the postnasal space. X-ray of sinuses indicated pansinusitis, which was subsequently adequately treated. Ten cubic centimeters of iodized oil were introduced into the base of each lung, and fluoroscopic and x-ray studies made following its introduction showed numerous bronchiectatic pocketings in both bases. Patient obtained symptomatic relief following the first introduction of oil. Treatments were continued at from three to five week intervals for a period of a year, at which time the chest had shown definite improvement. Patient continued to have recurrent pansinusitis in spite of adequate treatment, so was sent to a less rigorous climate where she remained for almost three years. She then returned to Richmond, and when seen had an acute respiratory infection. Fluoroscopic and x-ray studies showed the tracheobronchial tree entirely free of iodized oil, so treatments were resumed, and more than 100 c.c. of iodized oil were introduced within ten weeks. She made a most spectacular improvement.

Acute Respiratory Infection.—Mr. A. V., an executive, aged forty-five, was referred to us for treatment of an acute laryngitis, tracheitis, and bronchitis. He gave a history of what he described as sinus trouble since 1917, since when he has

been treated from time to time without permanent relief. On examination he was found to have an acute laryngotracheobronchitis. He also had an acute upper respiratory infection. X-ray of sinuses showed complete opacity of the left maxillary antrum. Ten cubic centimeters of iodized oil were introduced into the tracheobronchial tree, and patient was referred to an otolaryngologist for treatment of the sinuses, who irrigated the left antrum, removing considerable cystic fluid. The following day the patient reported that he had been completely relieved of chest symptoms following introduction of oil. He required no further treatment.

M. O. B., physician, aged sixty-five, consulted us complaining of an acute laryngitis, tracheitis, and bronchitis of three days' duration. He stated that his throat was so irritated that he was unable to rest. He was given 10 c.c. of iodized oil into the tracheobronchial tree. He experienced immediate and complete relief and except for slight expectoration he had no further symptoms. This patient had had no other treatment than the injection of oil.

Mr. L. C. J., aged fifty, was seen in consultation and found to have an acute tracheobronchitis which had not improved after five days of bed rest and medical treatment. He was given 10 c.c. of iodized oil. The following day his physician reported him definitely better and two days later the patient came to our office for another treatment. Following this treatment he was completely relieved.

SUMMARY

1. After five years' trial we are convinced that iodized oil has a definite therapeutic value in the treatment of most nontuberculous lung conditions.

2. While the literature contains a few contributions on its therapeutic value in chronic nontuberculous conditions we have been unable after careful search, to find specific reference to its use in acute respiratory lesions. Our experience has led us to believe that even better results are to be obtained in acute infections than in subacute or chronic.

3. It is contraindicated in acute tuberculosis, and in certain acute abscesses of the lung, but has some therapeutic value in lung gangrene.

4. It offers relief in certain patients with intrinsic asthma, afforded by no other drug or form of medication.

5. It is definitely, if not specifically, helpful in the treatment of many fungus lung diseases, where no other form of medication affords relief.

6. The field of iodized oil is broad and merits further study.

REFERENCES

1. Sicard, J. A., and Forestier, J.: Iodized Oil as Contrast Medium in Radioscopy, Bull. et mém. Soc. méd. d. hôp. de Par. **46**: 463-469, 1922.
2. Pritchard, S., Whyte, B., and Gordon, J. K. M.: Use of Iodized Oil in Diagnosis and Treatment of Bronchial Affections, J. A. M. A. **86**: 119-1123, 1926.
3. Ochsner, A.: Bronchography Following "Passive" Introduction of Contrast Media Into Tracheobronchial Tree, Wisconsin M. J. **25**: 544-554, 1926.
4. Cole, Dean B., and Whitehead, L. J.: Bronchograms in the Study of Pulmonary Disease, Va. Med. Mon., Nov., 1928.
5. Archibald, E., and Brown, L. A.: Dangers of Introducing Iodized Oil Into the Tracheobronchial System, J. A. M. A. **88**: 1310, 1927.

801 PROFESSIONAL BUILDING.

LABORATORY METHODS

THE CORRECTED ERYTHROCYTE SEDIMENTATION TEST*

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A METHOD BASED UPON CORRECTION FOR THE RED CELL COUNT

DESPITE the numberless reports concerning the utilization of the erythrocyte sedimentation test which have emanated from all parts of the world since the advent of this serologic test into clinical medicine in 1918,[1] it is surprising how incomparable are the results obtained and how few have been the attempts to free the reaction from certain inherent fallacies. It is surprising that the majority of workers have been content to read the distance which the erythrocyte column falls at the end of one or more hours without regard for certain extraneous factors which may so influence the S. R.,† as to render such a reading quite valueless. The S. T. would be in more general use were the method standardized, and a definite, corrected figure accepted as the normal.

Even the best methods which have been employed,[2 to 15] do not give results that can be accurately correlated. If the sedimentation test is to gain a permanent place among modern methods of clinical investigation, it must be simple and as free as possible from individual error.

I have been engaged in studies of the S. T. since March, 1927, and at the outset it was decided to revise the groundwork of the test in an effort to evolve a method which would be simple, inexpensive, and, at the same time free of the many factors of error which are present in the existing methods.

A CRITICAL STUDY OF EXPERIMENTAL CONDITIONS

Before embarking upon a description of the technic which I have employed, it seems necessary to amplify the foregoing statements by a brief discussion of certain factors which, although liable to cause fallacious S. R. readings, can readily be avoided.

When we undertake to perform a S. T. upon any patient we naturally wish to obtain a record of the changes which take place in the plasma of the individual to be investigated. The red cells can be considered as relatively inert bodies in themselves, merely serving as an index of the stability of the suspension which they make when intimately mixed up with the plasma as "blood."

If we approach the problem, keeping this idea well in mind, it is apparent that any extraneous factors must be kept constant as far as possible. When this object has been achieved we obtain a S. R. reading which is recorded in terms of a figure which depends solely upon factors inherent in the blood to be tested. Only in this

*From the Department of Medicine, the Johns Hopkins Medical School.
Received for publication, July 21, 1932.
†For the sake of brevity the terms S. T. and S. R. will be used throughout to indicate Sedimentation Test and Sedimentation Rate respectively.

way is it possible to perceive the subtle alterations in the plasma which take place when there is a departure from the normal standard of health.

The following are the cardinal factors which have to be considered:

A. INFLUENCE OF THE SHAPE OF THE SEDIMENTATION TUBE

Under this heading we include diameter, length, and angle of inclination of the tube. Many types of tubes were experimented with and the S. R. compared when the width of bore varied, when the height varied and even when the sides were of upright or inverted conical shape. It was found that when the tube is of very fine diameter the upper layer of red cells is ill-defined, due to their uneven fall, and a tube of less than 2 mm. internal bore should, therefore not be used. The most constant and even results were obtained in tubes whose length was 6 cm. and whose internal diameter was 6 mm. One cubic centimeter of blood in such a tube occupied a column 32.5 mm. long. The sides of the tube must be parallel and the lower extremity quite flat.

Inclination of the Tube.—This is a very important factor, for any deviation of the tube from the perpendicular leads to a marked acceleration of the S. R. Lundgren (loc. cit.) showed experimentally and Eric Ponder[16] demonstrated theoretically that the S. R., in inclined tubes, is much increased over that in vertical tubes. In fact in a 200 mm. column a divergence from the vertical of only 7.5° is sufficient almost to double the S. R. in a vertical tube.

This point is stressed because in a clinic where the S. T. was done as a routine, I have seen the blood specimens put aside to sediment in a haphazard way, sometimes vertically but more frequently casually leaning against the side of a cardboard box or in a rack with the tube slanting away from the perpendicular.

B. INFLUENCE OF THE ANTICOAGULANT

The most widely employed anticoagulant is a solution of sodium citrate in concentrations varying from 2 per cent to 5 per cent. As a general rule it is mixed with blood in the proportion of 1 c.c. of citrate to 4 c.c. of blood. It is fairly obvious that when such a solution as sodium citrate is added to blood, the suspension stability of which is about to be tested, it should be employed in such strength and quantity that it will, in itself, exert no appreciable influence upon the erythrocytes. Hence, an isotonic solution should be utilized. This point has only once been emphasized and that was by Westergren. An isotonic solution consists of 0.38 per cent sodium citrate and this should be added in minimal quantity so as to interfere with the S. R. as little as possible. It was found that 1 c.c. of a 3.8 per cent solution was quite sufficient to prevent 9 c.c. of human blood from clotting (at the same time maintaining isotonicity), whereas most authors have used double that quantity of citrate thereby diluting the blood unnecessarily. Moreover, Hesser[17] has shown that the autoagglutinating properties of serum may be very much reduced by the addition of a diluent to the extent of 1:2 and occasionally even at a dilution of 1:4.

In order to determine the relative merits or demerits of the various anticoagulants usually employed, I made many parallel experiments using citrated blood as the standard of comparison in each case. Hirudin, or leech extract would seem to be the most perfect anticoagulant, as it obviates the introduction of such compli-

cating factors as dilution and osmotic pressure changes that are inherent in the methods employing citrates or oxalates. However, the use of hirudin is necessarily restricted on account of its high price and in addition a number of my experiments were vitiated by the fact that clotting of the blood specimen commenced before the dried extract had time to act. Heparin also was tried but this, too, is expensive and does not seem to act as efficiently as sodium citrate. A solution of novarsenobillon was tried on several occasions only to be discarded as quite unsuitable.

Potassium oxalate crystals were employed fairly frequently, but owing to the fact that a slight degree of hemolysis occurred on several occasions, it was thought that the shape and surfaces of the red cells must be altered to some extent, thereby diminishing the agglutinability of the erythrocytes. The S. R. is more rapid and the rouleaux formation more extensive in heparinized and hirudinized blood than, in citrated or oxalated specimens.

C. INFLUENCE OF EXTERNAL TEMPERATURE

The room temperature at which the test is performed must be taken into account. Comparatively little mention of this factor is to be found in the literature until Gordon and Cohn[18] in 1928 and Rourke and Plass[19] in 1929 brought this point into prominence. By making dual experiments with specimens of the same blood, one tube of which has been kept for one hour in an incubator at 37° C. and another tube at room temperature (19° to 23° C.) during the same period, I have noticed that a much greater speed of fall was recorded at the higher temperature.

The optimum room temperature for the S. T. seems to be 19° to 23° C. Above or below this temperature, marked deviations of S. R. are found.

D. INFLUENCE OF THE RED CELL CONTENT

This factor has been largely neglected, and it is only during the past year or so that efforts have been made to correct for fluctuations in the erythrocyte count. Fahraeus[2] stated that the S. R. is faster in higher dilutions of blood, and a few others have confirmed this, notably Bonniger and Hermann[20] and Hubbard and Geiger.[21] Cooper[11] says that anemia does not affect the S. R., and Rubin[22] states that between the limits of 4,000,000 and 5,000,000 erythrocytes per c.mm. the red cell factor is of no appreciable importance. However, in 1929, Gram[23] published an article, wherein he pointed out the influence of the red cell content upon the S. R., and in this paper he described a method for correcting the S. R. by fixing a certain hemoglobin percentage (100 per cent) as the normal. On reference to a chart which he had constructed he was able to correct the S. R. on a basis of the hemoglobin content of the blood. Rourke and Ernstene[10] in 1930 published an account of a method which aims at correcting the S. R. upon a basis of cell volume. In a report describing the effect of anaphylactoid shock upon the S. R. in animals, Joltrain and I[26] in 1929 emphasized the importance of correlating the S. R. figures with the erythrocyte count.

When my work was still in the experimental stage it became obvious that the red cell count played an important part in determining the rate of fall of the sedimenting red cell column. For this reason, during the investigation of the first 300 patients, five tubes were always set up to sediment containing descending concentrations of erythrocytes from 5,000,000 to 1,000,000 cells per c.mm. By this means

a very careful and thorough comparison could be made between the sedimentation rates of the different specimens to be tested throughout a wide range of corpuscular concentration.

An easy method of calculating the necessary dilutions involved was obtained by modifying a simple formula given by Blacklock.[24] His formula indicates the amount of diluting fluid which it is necessary to add to one volume of fluid of percentage "x" in order to reduce that volume to the desired percentage "y."

It is as follows: $\frac{x}{y} - 1$ where x represents the original percentage and y the desired percentage.

This is readily applicable to blood by substituting the number of millions of red cells for a percentage. Thus, given 1 c.c. of citrated blood containing 5,400,000 red cells per c. mm., in order to dilute this so that the erythrocyte content becomes 5,000,000 per c.mm. it is necessary to add $\frac{5.4}{5.0} - 1$ c.c. = 0.08 c.c. of plasma. As a matter of convenience 10 c.c. of citrated blood was used, and after a red cell enumeration had been done, 5 c.c. of this blood was transferred to a clean glass container, the other 5 c.c. was centrifugalized, and the supernatant clear plasma pipetted off to be used as a diluent.

If 1 c.c. of blood, whose content is 5,400,000 red cells per c.mm., needs 0.08 c.c. of plasma, 5 c.c. will need 0.4 c.c. Therefore in such a case we add 0.4 c.c. of plasma to the 5 c.c. of blood, and there results 5.4 c.c. of blood whose erythrocyte content becomes 5,000,000 cells per c.mm. In other words, whatever the blood count may be, the million figure is noted and a similar quantity of blood in cubic centimeters is placed in the containing tube. To this is added a quantity of plasma, which is represented in fractions of a cubic centimeter, just as the red cell count figure below the million cipher is represented in fractions of a million.

Examples.—

1. Initial blood count 4,800,000 red cells per c.mm.: 4 c.c. of blood is placed in the containing tube and to it is added 0.8 c.c. of plasma. The tube now holds 4.8 c.c. of blood containing 4,000,000 red cells per c.mm.

2. Initial blood count 6,280,000 red cells per c.mm.: 6 c.c. of blood is placed in the containing vessel and 0.28 c.c. of plasma added to it. The vessel now holds 6.28 c.c. of blood whose count will be 6,000,000 red cells per c.mm.

The next step in the procedure is to mix this red cell suspension intimately by gentle inversion of the tube and, to distribute it among several sedimentation tubes so that by the addition of plasma varying concentrations may be obtained. Thus, to obtain 1 c.c. of blood which will contain 4,000,000 red cells per c.mm. from a suspension containing 5,000,000, it is necessary to place 0.8 c.c. of blood from the large tube into the sedimentation tube, and add to it 0.2 c.c. of plasma.

This dilution method works on the principle that if we have 5 c.c. of a suspension of red cells at 5,000,000 per c.mm., and we wish to make from it 5 c.c. of a suspension containing 4,000,000 red cells per c.mm. we must take 4 c.c. of the blood and add 1 c.c. of plasma. Similarly to make a 3,000,000 suspension we take 3 c.c. of the blood and add 2 c.c. of plasma.

If the original blood count is below 5,000,000 we can concentrate from any given red cell count by employing the same principles for the withdrawal of plasma.

Example.—If the erythrocyte count of the citrated sample is 4,500,000, then by addition of plasma to the red cell suspension in the proportion of 0.5 c.c. of plasma to 4.0 c.c. of blood we obtain a quantity of blood whose concentration is now 4,000,000 red cells per c.mm. To set up a 5,000,000 tube from this suspension one cubic centimeter is placed into each of two small tubes which are then centrifuged slowly for a few minutes so as to separate out a small quantity of supernatant plasma. From each of these two tubes, 0.2 c.c. of the clear plasma is removed by means of a small pipette thus leaving, in each tube, 0.8 c.c. of blood whose concentration is now 5,000,000 red cells per c.mm. After remixing one specimen, 0.2 c.c. of the red cell suspension is transferred from it to the other tube, and in this way we have a tube which contains 1 c.c. of blood whose concentration is 5,000,000 red cells per c.mm.

I have proved that this method is satisfactory by constantly checking and rechecking the red cell count at different stages in the process.

It was evident, from the first, that even small variations in the red cell content made a great difference in the S. R., for when a series of tubes was set up containing blood in descending concentration from 5,000,000 to 1,000,000 red cells per c.mm., the S. R. accelerated progressively in proportion to the increasing dilution.

These results were later confirmed, when in 1928, Hubbard and Geiger[21] described the effect upon the S. R. of blood by rough dilution and concentration of the specimen. They point out the difficulties of diluting or concentrating the blood by an accurate method so that a given number of red cells would be present. In their own words "It would not be possible to determine the S. R. and blood count * * * on a given patient and calculate what the rate would be for a different blood count."

A few examples will be quoted of cases taken at random from the list to indicate the influence exerted by the red cell content upon the S. R., and in the appended table there are included 50 cases which are representative of the total series investigated by the concentration and dilution method outlined.

CASE 1.—NORMAL. RED CELL COUNT 4,680,000

TUBE NO.	CONTENTS	S. R. IN 1 HOUR
5	5,000,000 red cells per c.mm.	1.0 mm.
4	4,000,000 red cells per c.mm.	6.0 mm.
3	3,000,000 red cells per c.mm.	10.5 mm.
2	2,000,000 red cells per c.mm.	20.0 mm.
1	1,000,000 red cells per c.mm.	27.5 mm.

CASE 18.—CARCINOMA OF CERVIX UTERI. RED CELL COUNT 4,270,000

TUBE NO.	CONTENTS	S. R. IN 1 HOUR
5	5,000,000 red cells per c.mm.	9.0 mm.
4	4,000,000 red cells per c.mm.	13.5 mm.
3	3,000,000 red cells per c.mm.	20.0 mm.
2	2,000,000 red cells per c.mm.	23.5 mm.
1	1,000,000 red cells per c.mm.	27.5 mm.

CASE 109.—ACTINOMYCOSIS OF CECUM. RED CELL COUNT 4,190,000

TUBE NO.	CONTENTS	S. R. IN 1 HOUR
5	5,000,000 red cells per c.mm.	16.0 mm.
4	4,000,000 red cells per c.mm.	19.0 mm.
3	3,000,000 red cells per c.mm.	22.0 mm.
2	2,000,000 red cells per c.mm.	25.0 mm.
1	1,000,000 red cells per c.mm.	27.5 mm.

Fig. 1 depicts better than any written description the effect of similarly treating blood taken from a normal individual.

From these examples it is readily seen what a notable influence the number of red cells has upon the S. R. in any particular case. It is just in the higher concentrations that a slight variation in the erythrocyte count has an important effect. On many occasions it has been remarked that in a patient who was found to be normal clinically, a rather rapid S. R. was obtained in the uncorrected specimen. The red cell count, however, was in the region of 4,000,000 and when the S. R. was measured in a tube which contained 1 c.c. of this same blood concentrated up to a 5,000,000 basis, the rate was so much retarded, thereby, as to fall within normal limits.

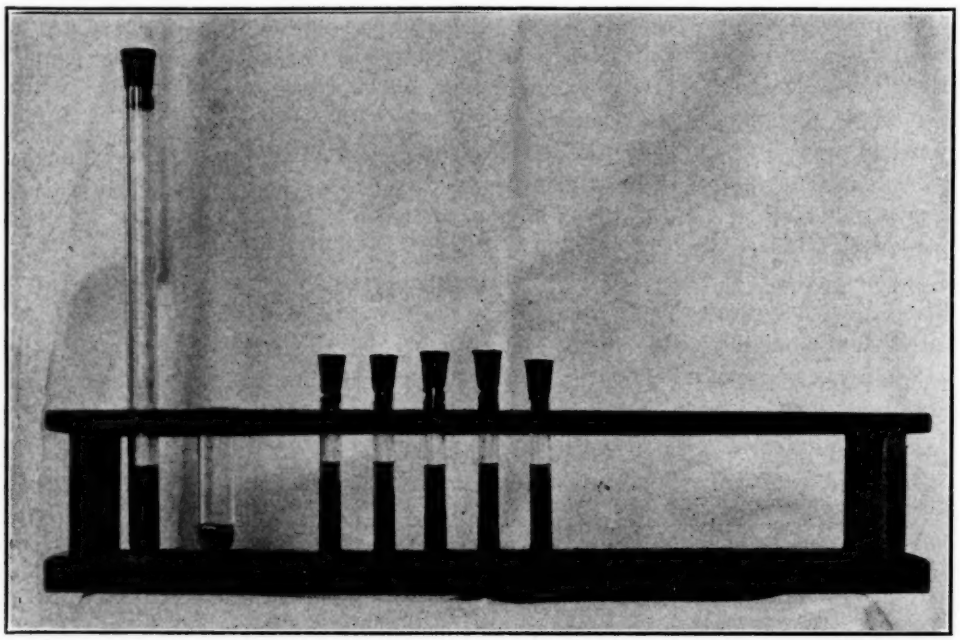

Fig. 1.—From left to right the tubes depicted contain: (1) Reserve supply of blood whose concentration is 5,000,000 red cells per c.mm. (2) Plasma for dilution. (3) S. tube containing 1 c.c. of blood whose red cell content is 5,000,000 per c.mm. (4) S. tube containing 1 c.c. of blood whose red cell content is 4,000,000 per c.mm. (5) S. tube containing 1 c.c. of blood whose red cell content is 3,000,000 per c.mm. (6) S. tube containing 1 c.c. of blood whose red cell content is 2,000,000 per c.mm. (7) S. tube containing 1 c.c. of blood whose red cell content is 1,000,000 per c.mm.

In the tubes of higher dilution the fall is much faster, a S. R. being obtained which is not quite proportionate to the extent of the dilution, but rather in excess of this ratio. In fact, it was almost constantly found that whether the blood was taken from a normal or a diseased patient, the S. R. in the 1,000,000 tube was 27.5 mm. Moreover in the 3,000,000 and 2,000,000 tubes the difference between the S. R. in normal and abnormal patients was so small, as compared with the wide margin in the 5,000,000 tube that the latter tube was used throughout as the standard of comparison.

Reference to the protocols displays this fact quite well. Whereas Case 1 had a S. R. of only 1.0 mm. in Tube 5, Case 109, frankly abnormal, showed a S. R. of 16.0 mm. On the other hand the S. R. in Tube 1 in both cases was the same, namely,

27.5 mm., a striking comparison. To a lesser extent a wider margin of difference is found to exist in Tubes 5 than occurs on comparison with the S. rates measured in Tubes 4.

There were further reasons for employing the 5,000,000 cell count as the fixed standard. In 1924 Hesser[17] made a serologic study of the red blood corpuscles, especially in relation to agglutination. The rate of sedimentation depends, as can easily be demonstrated, upon the degree of agglutination of the erythrocytes, whereby masses tend to fall faster than small agglomerates. Hesser showed that this agglutination and rouleaux formation occurs best at a high concentration of red cells. If the proportion of cells to plasma was 1:5 in a normal person the agglutination was moderate, although in a rapidly sedimenting blood it was more marked. If the blood was further diluted, the amount of agglutination diminished until at 1:40, in a normal individual, and at 1:80 in a diseased patient, all agglutination disappeared. Thus, in conditions of anemia, where the proportion of plasma to red cell content is increased, the degree of rouleaux formation is considerably diminished. This feature can be well observed if the process of sedimentation be carefully watched through a magnifying lens. In the tubes of higher concentration of red cells, there is a sharp line of demarcation between the upper level of the falling column of cells and the supernatant plasma. In the 3,000,000 tube and the tubes of blood of lower concentration, the large, heavier masses of cell agglomerates fall quickly, leaving in their wake a reddish haze the upper level of which is indistinct. This renders an accurate reading of the sedimentation height impossible. The red haze consists of slowly falling erythrocytes in clusters, which diminish in size to the upper extremity of the cloud, where cells are falling singly or in twos and threes.

Anemia, therefore, renders the S. T. valueless, unless it is corrected, for when a count of 1,000,000 cells per c.mm. is reached, maximal sedimentation with some packing of the cells has always occurred at the end of one hour, and before this interval of time the upper level of erythrocytes is too hazy for an accurate reading to be made.

In short, some degree of anemia is not infrequently encountered in disease processes, and if the effect of the pathologic condition upon the S. R. is to be studied, some attempt must be made to differentiate the effect of this primary factor from that of the secondary one. A great deal of space has been devoted to the red cell factor in the S. T., but the excuse for this lies in the importance of such a factor which cannot be, and indeed has not been so far, sufficiently emphasized.

PERSONAL TECHNIC

The first essential is that all glass tubes, pipettes, and syringes should be kept scrupulously clean and dry. A small flask of sterile, 3.8 per cent solution of sodium citrate is kept tightly corked so as to prevent evaporation, and thereby concentration. A fresh solution is frequently made up so that no deterioration is likely to ensue. The actual sedimentation tubes are 6 cm. long, 6 mm. in internal diameter, and the bottom of the tube is flat. A mark is cut on the glass at a height of 32.5 mm. from the base, this mark indicating a content of 1 c.c. Lately I have employed tubes graduated in half millimeters but for some time I was content to use a flat metal scale, recording in 0.5 mm., and by placing this alongside the tube it was a

TABLE I

THE EFFECT UPON THE S.R. OF DIFFERENT CONCENTRATIONS OF RED CELLS. THE S.R. WAS MEASURED AT THE END OF ONE HOUR IN A COLUMN OF BLOOD 32.5 MILLIMETERS HIGH

CASE NO.	SEX	DIAGNOSIS	RED CELL COUNT	SEDIMENTATION RATE IN TUBES OF DESCENDING RED CELL CONCENTRATION FROM 6 - 1 MILLION PER C.MM.					
				6 MILL.	5 MILL.	4 MILL.	3 MILL.	2 MILL.	1 MILL.
5	M	Normal	4,460,000		0	4.5	10.0	18.0	26.0
32	M	Normal	4,760,000		1.0	7.5	13.5	19.5	27.0
1	M	Normal	4,680,000		1.0	6.0	10.5	20.0	27.5
2	M	Normal	4,240,000		1.0	7.5	14.5	21.0	27.5
16	M	Normal	4,750,000		1.5	8.0	14.0	20.0	27.0
44	M	Normal	4,650,000		2.0	7.0	13.0	20.0	27.5
100	M	Normal	4,900,000	1.0	3.0	8.0	14.5	20.5	27.0
201	M	Normal	5,000,000		5.0	9.5	15.5	22.5	27.5
93	F	Normal: recovery from attack of pelvic cellulitis	4,000,000		0	2.5	7.0	12.5	25.0
17	F	Normal	4,940,000		1.5	7.5	14.0	21.0	27.5
31	F	Normal	4,070,000		2.5	8.0	15.5	21.5	27.5
38	F	Normal	4,550,000		3.0	7.0	13.0	21.0	27.5
51	F	Normal	5,180,000		4.0	9.5	15.0	22.0	27.5
52	F	Normal	4,850,000	1.0	4.5	8.0	15.0	21.0	27.5
64	F	Normal	5,000,000	0	4.0	10.0	15.5	21.5	27.5
62	F	Metrostaxis	3,980,000	1.0	4.5	9.0	15.0	21.0	27.5
205	F	Normal	5,000,000		5.0	11.0	16.5	22.5	27.5
13	F	Normal pregnancy, 9 weeks	3,730,000		0	5.0	12.5	19.5	27.5
56	F	Pregnancy, 18 weeks	4,110,000		4.0	9.5	14.5	21.0	27.5
68	F	Pregnancy, 12 weeks	4,200,000		8.5	13.0	17.5	23.5	27.5
47	F	Ruptured ectopic gestation	3,740,000	5.0	9.0	14.0	18.5	22.5	27.5
28	F	Pregnancy, 38 weeks	3,880,000		10.0	14.0	18.0	22.5	27.5
12	F	Pregnancy, 24 weeks	4,780,000		10.0	14.5	19.0	23.5	27.5
58	F	Pregnancy, 38 weeks	3,970,000	6.5	11.0	15.5	19.5	23.5	27.5
45	F	Pregnancy, 12 hours post-partum	4,710,000		12.0	16.0	20.0	24.0	27.5
195	F	Pregnancy with toxemia, 36 weeks	4,880,000	13.5	16.0	19.0	22.0	25.0	28.0
53	F	Chronic pyosalpinx	4,820,000	12.0	16.0	19.0	21.0	25.5	28.0
145	F	Acute pyelitis	5,990,000		9.0	14.0	18.5	23.0	27.5
120	M	Chronic osteomyelitis	5,140,000		16.0	19.0	27.0	24.5	28.0
131	F	Post operative hematoma	3,140,000	12.5	15.5	18.5	22.0	25.0	27.5
137	F	Typhoid fever	4,440,000		12.5	16.0	20.0	24.0	27.5
140	F	Convalescent typhoid fever	4,700,000		7.5	13.0	18.5	23.5	27.5
94	M	Tbc. pleural effusion	4,470,000	8.5	13.5	16.5	20.0	24.0	28.0
118	F	Tbc. pleural effusion	5,330,000		14.5	18.0	22.0	24.5	28.0
15	F	Tbc. peritonitis	4,140,000		11.0	15.0	18.5	22.5	27.5
108	M	Actinomycosis of cecum	4,000,000		12.5	15.5	20.0	25.0	27.5
109	M	Actinomycosis of cecum	4,190,000	12.5	16.0	19.0	22.0	25.0	27.5
147	F	Subacute nephritis	3,020,000		13.5	17.0	21.5	24.0	27.5
163	M	Acute glomerular nephritis	5,200,000		15.5	18.0	21.5	25.0	28.0
86	F	Necrotic retroperitoneal sarcoma	3,360,000		12.5	16.0	20.0	23.5	27.5
134	M	Hypernephroma	3,960,000		13.0	17.0	21.0	25.5	27.5
50	F	Carcinoma peritonei with ascites	3,660,000	5.0	9.5	14.0	18.5	23.0	27.5
141	F	Carcinoma peritonei with ascites	4,370,000		13.0	16.5	20.0	23.5	27.5
116	M	Carcinoma of oesophagus	5,500,000		15.0	18.5	21.5	25.0	28.0
99	F	Carcinoma of stomach	5,060,000	13.5	16.5	19.5	22.0	25.0	28.0
127	F	Carcinoma of stomach	2,750,000		16.0	19.0	22.0	24.5	28.0
101	M	Carcinoma of pancreas	2,730,000		11.5	15.0	19.5	23.5	27.5
110	F	Carcinoma of liver	3,500,000		14.5	17.0	21.0	24.5	27.5
19	F	Paget's disease of breast	4,930,000		11.0	15.0	18.5	23.5	27.5
21	F	Scirrhous carcinoma of breast	5,110,000		8.5	13.5	19.0	23.0	27.5

simple matter to read off the height of clear supernatant plasma. A special rack was designed to hold the tubes during sedimentation. There are three rows of holes in the upper platform, and corresponding slots in the base, so that the tubes fit snugly, and are held absolutely vertical during sedimentation.

At one end of the rack are some larger holes to take the larger reservoir tube of blood, and another tube for the plasma with which the dilutions are made. For the purposes of my investigation I had, for a long time set up a series of five sedimentation tubes containing blood in descending concentration from 5,000,000 to 1,000,000 red cells per c.mm., and in such instances 10 c.c. of blood was necessitated. Later, however, it was found that 4.5 c.c. of blood would suffice, in order to set up one tube with a content of 5,000,000 cells per c.mm.

By the usual method, 4.5 c.c. of blood is withdrawn from an antecubital vein, taking great care not to produce an unnecessary degree of venous stasis, otherwise in a rapidly sedimenting blood some erythrocyte fall *in vivo* will occur (2 and 25).

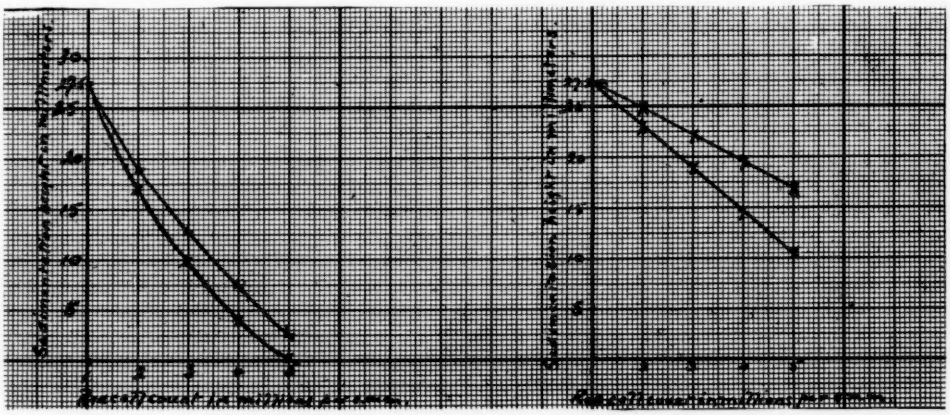

Fig. 2. Fig. 3.

Fig. 2.—Illustrates the sedimentation curves obtained from two normal individuals.
Fig. 3.—Illustrates the sedimentation curves obtained from two pathologic cases.

This specimen is rapidly introduced into a tube and intimately mixed with 0.5 c.c. of sodium citrate solution already contained therein. A red cell count on this sample is now made, so that it will be known how to divide, approximately, the 5 c.c. specimen for dilution or concentration. It is usually expedient to place 2 c.c. of blood in a small tube, and to centrifuge the other 3 c.c. in order to obtain plasma with which to dilute the specimen to be tested. The object is to have at least 2 c.c. of blood, the red cell content of which after appropriate addition of plasma, will register an even million figure, i. e., 6,000,000, 5,000,000, 4,000,000, etc. From this suspension we can, by employing the method just described, readily abstract a sample of blood for a S. T. so that the sedimentation tube will contain 1 c.c., the content of which is 5,000,000 red cells per c.mm. The sedimentation tube is now corked, and an even suspension of blood obtained by inverting and rotating the tube about its long axis. If any air bubbles remain on the surface, they must be burst by the application of a heated glass rod before sedimentation is allowed to proceed. The sedimentation height or height of clear plasma lying above the column of red cells is then measured at the end of one hour.

A MODIFIED METHOD

In certain cases it is desirable to utilize small quantities of blood: for example when frequent repetition of the test is necessary during the observation of chronic pathologic conditions, such as pulmonary tuberculosis, or when a S. T. is performed upon infants and the longitudinal sinus route is chosen. For this purpose a method has been devised, which is simple and rapid, so that a large number of sedimentation tubes can be set up at once. This method arose out of the observation referred to that, whatever the S. R. in a blood specimen at a high concentration might be, a height of fall of 27.5 mm. was almost always attained in the 1,000,000 tube at the end of one hour. During the time when five tubes of descending red cell concentration were set up as a routine, a graphic record was made in many of the cases. Figs. 2 and 3 illustrate typical findings in normal (Fig. 2) and abnormal individuals (Fig. 3).

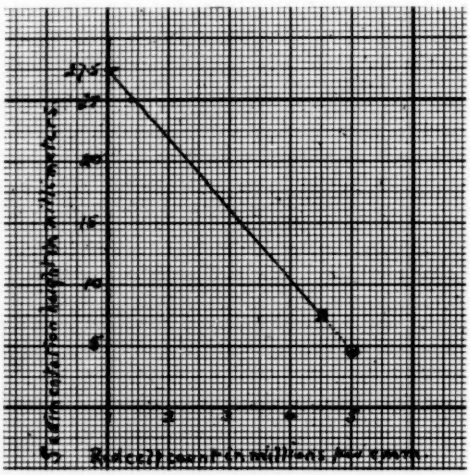

Fig. 4.—Depicts the procedure for obtaining the corrected S. R. when the modified technic is employed.

The S. R. values are plotted upon the graph as ordinates and the red cell counts are abscissae.

In a normal individual, the curve obtained is almost a straight line. When the S. R. is more rapid the curve described is always a straight line as shown in Fig. 3. From a consideration of these curves it is seen that, given a tube of blood at a concentration of 4,500,000 cells per c.mm., it is only necessary to measure the S. R. of this specimen, record the S. R. figure upon such a graph opposite the red cell count number of the blood, join the point obtained with the 27.5 mm. mark and continue the straight line until it cuts the 5,000,000 ordinate. At the point where this line crosses the 5,000,000 perpendicular, we read off the S. R. which would obtain if that specimen of blood were concentrated up to a 5,000,000 red cell count.

Red Cell Count.—

$$4{,}500{,}000 : \frac{\text{S. R. in one hour.}}{7.5 \text{ mm.}} \qquad \text{Corrected S. R. (to a 5,000,000 basis)} \quad 4.5 \text{ mm.}$$

Technic.—A dry 1 c.c. "Record" syringe is taken and 0.1 c.c. of 3.8 per cent sodium citrate solution sucked into it. Exactly 0.9 c.c. of blood is drawn into the

syringe from an arm vein; the citrate and blood are intimately mixed and the contents of the syringe evacuated into an ordinary 1 c.c. sedimentation tube. A hemocytometer pipette is introduced into the suspension, and enough blood withdrawn for a red cell enumeration, whereupon sedimentation is allowed to proceed in the usual way. If it is necessary to set up several specimens, they may all be placed in the rack to sediment, and the blood counts are performed at any time after the sedimentation height has been read, to suit the operator's convenience.

DISCUSSION

For more than five years the method described has been employed by the author during the investigation of a wide range of cases, culled from medical, surgical, gynecologic and obstetric wards. A full account of some of these findings is being reported elsewhere.[27] The number of experiments upon the blood of human beings and animals runs into several thousands, and records to hand amount to upwards of a thousand S. tests upon more than 500 patients. This includes a series of 50 cases of pulmonary tuberculosis, who were observed over a period of four months during their stay in a tuberculosis sanatorium. Repeated S. tests were performed upon these patients, and graphic records were kept of the S. R., weight, and temperature. The importance of the corrected S. R. reading is well seen in such an investigation. Not only does the red cell count differ from patient to patient, but during the course of a chronic disease notable fluctuations in the erythrocyte count are observed, and a false impression of the pathologic condition is obtained if suitable corrections are not made. It was frequently remarked that a sudden rise in the erythrocyte count occurred during a flare-up of the tuberculous process or immediately following collapse therapy. In such instances, and these are but two examples chosen from many others that could be quoted, the true state of affairs is faithfully reflected by the corrected S. R. figure, whereas the uncorrected figure gives an entirely erroneous picture. When the corrected S. R. is utilized, the test becomes more accurate and delicate, enabling one to detect changes in the suspension stability of the blood without costly apparatus or extraordinary skill on the part of the observer. Thus the scope of the test is widened and can be utilized in certain specialized ways. In the early diagnosis of tuberculosis, it may be employed as a *specific* diagnostic test, when used in conjunction with the injection of an infinitesimal dose of tuberculin. Another avenue of research is opened in the investigation of hepatic dysfunction by employing the S. T. and noting the changes in the S. R. after a protein-containing meal. Without corrected S. R. figures such special tests as these would not be feasible.

Of the two methods described, the concentration and dilution method, in which one tube of blood is set up containing 5,000,000 red cells per c.mm., is necessarily somewhat more accurate than the graphic modification, and this applies especially when the erythrocyte count is in the region of 3,000,000 or less. In the event of such a low cell count, it is urged that the specimen of blood should be concentrated, and the S. R. measured in a tube containing 1 c.c. of blood at a concentration of 5,000,000 cells per c.mm.

The normal one hour S. R. figures obtained by the methods described are 0.0 mm. to 5.5 mm. with an average of 2.5 mm. in men, and 0.0 mm. to 5.5 mm. with an average of 3.5 mm. in women.

CONCLUSIONS

1. A new procedure is described which endeavors to standardize the erythrocyte sedimentation test and thus eliminate certain fallacies which are inherent in many methods hitherto employed.

2. By this technic, which is simple, rapid, and inexpensive, an accurate comparison of the S. R. may be made in different blood specimens. The S. R. figure obtained is not influenced by any external factors but is a true reflection of the effect of abnormal states of the plasma upon the suspension stability of a fixed number of erythrocytes.

3. The method described necessitates the employment of 4.5 c.c. of blood which is added to 0.5 c.c. of a 3.8 per cent solution of sodium citrate. A red cell count is made of the citrated specimen and then, by means of a simple procedure, the degree of dilution or concentration is calculated which is necessary to bring the sample of 1 c.c. of blood to 5,000,000 red cells per c.mm.

4. The distance that the red cell column falls is measured in millimeters at the end of one hour, and the recorded result is the corrected S. R. *Thus in every case investigated, the S. R. is always spoken of in terms of the distance fallen in an hour by a column of red cells in a tube of 6 mm. diameter containing 1 c.c. of blood at a concentration of 5,000,000 red cells per c.mm.*

5. When it is desirable to take only a small quantity of blood, a modification of this method may be employed which is more rapid and requires merely 1 c.c. of citrated blood. When the S. R. of this specimen is measured, and a blood count made, the S. R. figure is corrected to a 5,000,000 red cell basis by means of a graph.

6. This method is not quite so accurate as the previous one, and should not be used when the red cell count lies below 3,000,000. However, it is readily adaptable to the repeated study of any case over a prolonged period of time and especially to the study of children.

7. The utilization of the corrected sedimentation test over a period of more than five years has led me to repose great confidence in the results obtained which have amply justified its adoption.

NOTE: The special sedimentation tubes described in this paper are being made by Arthur H. Thomas Co., Philadelphia.

REFERENCES

1. Fahraeus, R.: Agglutination in Blood, Hygiea **80**: 369, 1918.
2. Fahraeus, R.: Suspension Stability of Blood, Acta Med. Scandinav. **55**: 3, 1921.
3. Linzenmeier, G.: The Sedimentation Rate of Human Red Cells, Pflug. Arch. f. d. ges. Physiol. **181**: 169, 1920.
4. Westergren, A.: Suspension Stability of Blood in Pulmonary Tuberculosis, Acta Med. Scandinav. **54**: 247, 1921.
5. Katz, G.: Sedimentation Speed of Erythrocytes in Tuberculosis, Ztschr. f. Tuberk. **35**: 401-424, 1922.
6. Goodell, H., and Zeckwer, I. T.: Sedimentation Rate of Erythrocytes, Am. J. M. Sc. **169**: 209-216, 1925.
7. Forman, J.: Sedimentation Rate of Erythrocytes; Corpuscle Volume; and Icterus Index, With Simple Method of Combining All Three Tests, J. LAB. & CLIN. MED. **12**: 373-378, 1927.
8. Lundgren, R.: A Study of the Physical Nature of the Sedimentation of Blood Corpuscles, Acta Med. Scandinav. **67**: 63-105, 1927.
9. Gram, H. C.: The Correction of the Sedimentation Rate for the Cell Volume Percentage (haemoglobin) and the Normal Limits of the Sedimentation Reaction, Acta Med. Scandinav. **68**: 108-122, 1928.
10. Rourke, M. D., and Ernstene, A. C.: Method for Correcting Erythrocyte Sedimentation Rate for Variations in Cell Volume Percentage of Blood, J. Clin. Invest. **8**: 545-559, 1930.

11. Cooper, H. N.: Sedimentation Rate of Red Blood Cells, J. LAB. & CLIN. MED. **11**: 615-623, 1926.
12. Plass, E. D., and Rourke, M. D.: Effect of Venous Stasis on Proteins of Blood Plasma and on Rate of Sedimentation of Red Blood Corpuscles, J. LAB. & CLIN. MED. **12**: 735-742, 1927.
13. Gilbert, A., and Tzank, A.: Measurement of the Sedimentation Rate of Blood, C. R. Soc. de Biol. **88**: 873-875, 1923.
14. Balachowsky, S.: The Immediate Post Prandial Variations of the Sedimentation Rate of Erythrocytes, Presse méd. **33**: 629-631, 1925.
15. Cutler, J.: Finger Puncture Method for the Blood Sedimentation Test, Am. J. M. Sc. **173**: 687-694, 1927.
16. Ponder, E.: On Sedimentation and Rouleaux Formation, Quart. J. Exper. Physiol. **15**: 236-252, 1925.
17. Hesser, S.: Serological Studies of Human Red Corpuscles, Acta Med. Scandinav. **61**: 1-94, 1924.
18. Gordon, M. D., and Cohn, D. J.: Effect of External Temperature on Sedimentation Rate of Red Blood Corpuscles, Am. J. M. Sc. **176**: 211-214, 1928.
19. Rourke, M. D., and Plass, E. D.: Investigation of Various Factors Which Affect Sedimentation Rate of Red Blood Cells, J. Clin. Invest. **7**: 365-386, 1929.
20. Bonniger, M., and Hermann, W.: Sedimentation Speed and Volume of Erythrocytes, Klin. Wchnschr. **2**: 744-745, 1923.
21. Hubbard, R. S., and Geiger, H. B.: Anaemia as Factor in Sedimentation Time of Erythrocytes, J. LAB. & CLIN. MED. **12**: 322-326, 1928.
22. Rubin, E. H.: Relation of Erythrocyte Sedimentation Reaction to Ability of Flocculation of Plasma and Serum, Arch. Int. Med. **37**: 848-860, 1926.
23. Gram, H. C.: Sedimentation Rate of Red Blood Corpuscles in Various Internal Diseases and Result of Correction of This Value for Variations of Haemoglobin Percentage, Acta Med. Scandinav. **70**: 242-275, 1929.
24. Blacklock, E.: Percentage and Fractional Dilutions, Lancet **1**: 377, 1921.
25. Ploman: The Ophthalmoscopic Demonstration of the Sedimentation of Erythrocytes, Ann. d'Oculistique **157**: 1920. Quoted by Fahraeus.[2]
26. Joltrain, E., and Walton, A. C. R.: The Sedimentation Rate of Erythrocytes During the Course of Peptone Shock, Rev. de Méd., Paris **46**: 543-563, 1929.
27. Walton, A. C. R.: The Sedimentation Rate of Erythrocytes, a Clinical and Experimental Study, Quart. J. Med. **2**: 79-117, 1933.

A CLINICAL METHOD FOR SERUM PROTEIN*

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RECENT researches have called the attention of clinicians to the importance of the determinations of serum protein as a routine clinical measure. Heretofore this determination has been performed largely in research laboratories, and the methods have been difficult and cumbersome for the clinical laboratory technician. There is a demand now that this determination be placed on the basis where it can be carried out by the technician in the average office and hospital laboratory. The following is an attempt to make the present knowledge on the subject available for use in routine clinical work.

The steps are as follows:

1. Determine the total nonprotein nitrogen of the serum;
2. Determine the total nitrogen of the serum;
3. The difference between the two is the protein nitrogen;
4. The protein nitrogen multiplied by 6.25 gives the protein.

Nitrogen is most conveniently determined by Kjeldahl digestion and Nesslerization. This method was developed on a micro basis so that small quantities of

*Received for publication, June 12, 1932.

serum may be utilized. For the digestion, a micro-Kjeldahl flask of 25 c.c. capacity was made for the purpose by the Fischer Scientific Co., of Pittsburgh.

Nonprotein Nitrogen.—Remember to use the serum instead of the customary whole oxalated blood.

3 c.c. of serum in a small flask
21 c.c. of distilled water
3 c.c. of 10 per cent sodium tungstate
5 c.c. ⅔ Normal sulphuric acid

Mix and allow the mixture to stand for ten minutes.

Filter or centrifuge;

Take 10 c.c. of the filtrate or supernatant fluid, which is equivalent to 1 c.c. of serum; place in a micro-Kjeldahl flask;

Add a bead or a piece of brick or gravel to prevent bumping;

Add 1 c.c. of the digestion mixture.

Digest according to the Kjeldahl method.

When digestion is complete, drop a piece of litmus paper into the flask, and then add 10 per cent sodium hydroxide up to the neutral point. Be careful not to go past the neutral point, on account of the danger of driving off nitrogen in the form of ammonia with an excess of alkali. This neutralization with alkali is necessary because if the mixture is too strongly acid to be nesslerized, it neutralizes all the alkali in the Nessler's solution and fails to bring out the required yellow color.

If the liquid is not clear, it must be diluted to a definite volume, say 25 c.c., filtered or centrifuged, and an aliquot part, say one-half of the filtrate or supernatant fluid taken; and the factor *2* introduced into the calculation.

Nesslerize and read in the colorimeter.

In this laboratory where the Klett glass standard colorimeter is used, the calculation is as follows:

Multiply the reading by the glass-correction, 0.95; this gives *R*.

15/R × dilution (× 2 if clearing of the digested fluid is necessary and aliquot parts taken) gives the milligrams of nitrogen per 100 c.c. of serum. "Dilution" refers to the total number of c.c. of nesslerized fluid. This is a short cut; the full expression is:

15/R × dilution 100 × 100 equals mg. per 100 c.c. serum.

Where a Duboseq type of colorimeter is used, with a standard containing 1 mg. nitrogen per 100 c.c. of nesslerized fluid, the calculation is:

S/U × dilution equals mg. of nitrogen per 100 c.c. serum.

Total Serum Nitrogen.—Place 0.05 c.c. serum in the micro-Kjeldahl flask. This amount may either be measured in a Kahn pipette, in which case it must be delivered below the surface of the distilled water with which it is to be diluted; or 0.5 c.c. may be diluted to 10 c.c. with distilled water, and 1 c.c. of this used for the determination;

3 or 4 c.c. of distilled water
1 c.c. of digestion fluid

Digest as in the case of the total nonprotein nitrogen. Neutralize as before with 10 per cent sodium hydroxide.

Nesslerize and read in the colorimeter.

Calculation for Klett colorimeter: Reading × 0.95 glass-correction gives *R*. 15/R × dilution × 20 (× 2 if filtering was necessary) equals mg. total nitrogen per 100 c.c. serum:

For a Duboseq type of colorimeter using 1 mg. nitrogen per 100 c.c. of nesslerized standard:

S/U × dilution × 20 (× 2 if filtration is needed) equals mg. total nitrogen per 100 c.c. serum.

Digestion Mixture: To 50 c.c. of 5 per cent copper sulphate solution add 300 c.c. of 85 per cent (syrupy) phosphoric acid and mix. Add 100 c.c. of concentrated sulphuric acid and 25 gm. potassium sulphate; mix thoroughly.

For use, dilute with equal parts of water. Keep well stoppered and protected from light.

Digestion flasks after use may be cleaned with strong alkali.

Calculation of Serum Protein.—From the total nitrogen subtract nonprotein nitrogen. The figure gives the number of milligrams of protein nitrogen per 100 c.c. of serum. Multiplying this by 6.25 gives the number of milligrams of protein per 100 c.c. of serum. As serum protein is frequently expressed in percentage, this last figure may be divided by 1000 if the percentage of protein is desired.

Acknowledgment is made to Miss Violet Payne, technician, for the large amount of detail work necessary to develop and check the above method.

STUDIES IN THE SEROLOGY OF SYPHILIS*

X. Precipitation Tests for Syphilis With Spinal Fluids

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WHEN, following the development of the Kahn test, precipitation tests for syphilis began to be generally adopted, it was naturally expected that they would prove as serviceable in the examination of the spinal fluid as they soon proved to be with serum. It was, however, found by many serologists who attempted parallel tests, that whereas precipitation tests with serum were usually more sensitive than the Wassermann reaction, they were distinctly less sensitive when applied to spinal fluids. This inferiority was particularly striking if the Wassermann technic involved the use of a sensitive antigen, ice box incubation, and, most important, the use of large quantities of spinal fluid, 2 to 10 times the amount of serum used in the ordinary serum Wassermann.

The author recently described a simplified precipitation test for syphilis,[1] which in over 26,000 serum tests was found to be considerably more sensitive than either the Wassermann or Kahn reactions and, because of the ease of reading results, was considered to be more reliable than other precipitation tests currently used. When an attempt was made to apply this test to spinal fluids, it was found

*From the Syphilis Division of the Department of Medicine, Johns Hopkins Medical School. Received for publication, June 1, 1932.

that the spinal fluid Wassermann was as far superior to the precipitation test as the latter was to the Wassermann in the examination of serum. The results obtained in 614 spinal fluids,* most of them from patients previously diagnosed and treated as syphilitic, are listed in Tables I and II.

TABLE I

ANALYSIS OF RESULTS IN 614 SPINAL FLUIDS*

WASSERMANN REACTION IN								FLUIDS	PRECIPITATION TEST (1 C.C.)	
C.C. 1.0	0.8	0.6	0.4	0.2	0.1	0.05	0.02		+	±
0	0	0	0	0	0	0	0	517	(1)**	0
±	0	0	0	0	0	0	0	5	0	0
+	0	0	0	0	0	0	0	3	0	0
+	+	0	0	0	0	0	0	2	0	0
+	+	±	0	0	0	0	0	2	0	0
+	+	+	0	0	0	0	0	2	1	0
+	+	+	±	0	0	0	0	2	0	1
+	+	+	+	0	0	0	0	15	4	5
+	+	+	+	±	0	0	0	4	0	0
+	+	+	+	+	0	0	0	4	4	0
+	+	+	+	+	±	0	0	10	6	1
+	+	+	+	+	+	0	0	8	6	1
+	+	+	+	+	+	±	−0	8	7	1
+	+	+	+	+	+	+	0	6	6	0
+	+	+	+	+	+	+	±	26	25†	0
+	+	+	+	+	+	+	+			

Total	Wassermann Results			Precipitation Results		
	+	±	0	+	±	0
	92	5	517	59	8	547

*In this, as in the following tables of this paper,
+ indicates a definite positive reaction with no hemolysis;
0 indicates a definite negative reaction, with complete hemolysis; and
± indicates a doubtful reaction, with partial hemolysis.

**The negative Wassermann reaction was due to some technical error, the reaction being positive upon repeated test.

†The negative precipitation test was due to some technical error, the reaction being positive upon repeated test.

Neither of these fluids is included in the discussion of discrepant Wassermann and precipitation tests.

TABLE II

ANALYSIS OF 96 POSITIVE AND DOUBTFUL SPINAL FLUIDS

NO. OF FLUIDS	PRECIPITATION TEST	WASSERMANN	AVERAGE REAGIN TITER OF THE FLUIDS*
5	0	±	<1
24	0	+	3
8	±	+	4½
59	+	+	20-25

*Wa—positive in 1 c.c. = 1 unit; Wa—positive in 0.2 c.c. = 5 units; etc.

*Fifteen anticomplementary spinal fluids, 11 of which yielded a positive precipitation test, are not included in the following discussion.

PROTOCOL 1

Wassermann.—The Wassermann technic was exactly that described in the following paper of this series,* save that instead of using 0.1 and 0.05 c.c. as in the serum test, the spinal fluids were tested as outlined in the following table, in quantities of as high as 1 c.c.

Spinal fluid, c.c.	1.0	0.8	0.6	0.4	0.2	0.1	0.05	0.02	0.01	0.005
Complement, 1:12 c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0.02	0.2	0.2	0.2
Antigen 1:200, c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Incubation for 4 hours at 0°-6° C., followed by ½ hour at 37° C.

Precipitation Test.—Exactly as described in preceding papers of this series,*† save that 1 c.c. of spinal fluid was used instead of 0.4 c.c., as in the serum test, the quantity of antigen suspension remaining the same (0.04 c.c.). As is stated in the text, it has since been found that an even smaller antigen: fluid ratio (0.02 c.c. of antigen suspension + 2 c.c. of fluid) results in a more sensitive reaction.

As is there shown, the new precipitation test detected only 67 of the 98 Wassermann-positive or doubtful fluids. The Kahn test proved even less sensitive. Moreover, the outcome of the precipitation test depended upon the amount of reagin present in the fluid, and could usually be predicted from the "strength" of the positive Wassermann reaction. As outlined in Protocol 1, the Wassermann test employed involves the use of graded quantities of fluid, beginning with 1 c.c. of whole fluid, and decreasing to 0.005 c.c. (1, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05, 0.025, 0.01, 0.005). Whenever the Wassermann was positive with as little as 0.05 c.c. of fluid, the precipitation test (carried out with 1 c.c. fluid) was usually also positive. As the amount of the reagin in the fluid decreased, however, negative precipitation tests began to appear. Thus, in spinal fluids which were Wassermann positive only down to 0.4 c.c., positive precipitation tests were the exception rather than the rule; while fluids containing so little reagin as to be Wassermann positive only with 0.6, 0.8, or 1 c.c. of fluid, almost always gave a negative precipitation test. Roughly, the Wassermann proved to be 2½ to 3 times as sensitive as the precipitation test, requiring only 2/5 to 1/3 as much reagin in order to yield a positive reaction.

This has been the experience of most serologists who have attempted to apply precipitation tests to spinal fluid. Recognizing the difficulty, Kahn developed a new procedure for the examination of spinal fluids, involving the precipitation of the spinal fluid globulin (containing the active reagin) by approximately half saturation with $(NH_4)_2SO_4$, redissolving the precipitate in a minimum volume of physiologic salt solution and using this concentrated globulin solution in a precipitation test.[3] In this laboratory, however, this procedure has proved entirely unsatisfactory, and for two distinct reasons.

Even with reasonable care in the removal of the supernatant $(NH_4)_2SO_4$ solution after the centrifuging of the globulin precipitate, so much $(NH_4)_2SO_4$ remains in the precipitate that when the antigen suspension is added to its solution, there is a nonspecific aggregation, due to excess electrolyte, which simulates the aggregation due to reagin.

A more serious objection is the fact that the procedure usually yields no globulin precipitate in the very spinal fluids in which the ordinary precipitation test is negative and the Wassermann positive, that is, the very fluids in which the

*Page 684, this issue.
†J. LAB. & CLIN. MED. **17**: 787, 1932.

procedure should find its application. Of the 24 fluids in our series falling into this category, all but 5 gave a negative or weakly positive Pandy test, and a flat or nearly flat mastic curve; in other words, at least 75 per cent of these positive fluids contained so little globulin that no significant precipitate would form upon half saturation with $(NH_4)_2SO_4$. True, full saturation of such fluids does yield a precipitate, consisting almost entirely of the normal spinal fluid protein (25 mg. per cent or less), plus any reagin or globulin which may have been present; but such a saturated solution has so high a specific gravity that the protein will not sediment clearly upon centrifuging. Moreover, the salt content of any slight sediment obtained is so high as to preclude the ordinary precipitation test.

Nor can it be said that these fluids in which the Wassermann was weakly positive, the precipitation test negative, and the protein content either normal or only slightly increased, represent false positive Wassermann reactions; for three-fourths of these cases presented clinical evidence of central nervous system involvement (Table III).

Several other methods of concentrating spinal fluid reagin were then attempted in this laboratory, such as the precipitation of the globulin with CO_2, the addition of normal serum to the spinal fluid before precipitation with 50 per cent $(NH_4)_2SO_4$, and the centrifuging of the lipoid particles from spinal fluid at varying P_H. None of these proved satisfactory.

A significant increase in the sensitivity of the new precipitation test with spinal fluid was finally attained by using much less antigen in proportion to the spinal fluid than is used in the serum test. The latter test calls for a 1:10 antigen: serum ratio; by decreasing this ratio to 1:100 (2 c.c. of spinal fluid + 0.02 c.c. of antigen suspension), a considerable increase in sensitivity was effected, without however, attaining the sensitivity of the Wassermann reaction.

The Wassermann reaction therefore remains the method of choice in the examination of spinal fluids. Until some method has been found of increasing the sensitivity of the precipitation test in fluids, the Wassermann cannot be entirely replaced by precipitation tests, no matter how great their technical simplicity, reliability or sensitivity with serum.

Of perhaps only academic interest is the explanation of this puzzling difference in the reactivity of serum and spinal fluid. Why does a precipitation test give a higher proportion of positive reactions with serum than the Wassermann reaction, but less with spinal fluids?[4 to 8]

The answer is to be found in the well known fact that complement fixation is inhibited by serum, as illustrated in the following simple experiment. If one titrates a known strongly Wassermann-positive serum by both the Wassermann and a precipitation test, using a constant quantity of serially increasing serum dilutions, one usually finds that the Wassermann titer is higher; that is, that complement fixation as such is a more delicate test for reagin than the aggregation reaction. If this same experiment is repeated, diluting the syphilitic serum with normal serum instead of with physiologic salt solution, one observes a striking inhibition of fixation, the apparent titer falling to 1/8 and even 1/16 of its original value: the precipitation reaction, however, is practically unaffected by the serum. Clearly, some substance present in normal serum inhibits fixation, and masks

the reagin. The active inhibiting component has been found in this laboratory to be associated with the albumin fraction of the serum protein. Possibly, this forms a protective film around the lipoid-reagin particles, and prevents the fixation (adsorption) of complement. Be that as it may, given a serum con-

TABLE III

ANALYSIS OF 29 FLUIDS WITH A POSITIVE OR DOUBTFUL WASSERMANN REACTION, AND A NEGATIVE PRECIPITATION TEST

	WASSERMANN REACTION						PANDY	MASTIC CURVE	TOTAL PROTEIN MG. %	CLINICAL EVIDENCE OF NEUROSYPHILIS
	1	0.8	0.6	0.4	0.2	0.1				
1	±	0	0	0	0	0	0	0	25	Sluggish reflexes
2	±	0	0	0	0	0	++	232210	50	Argyll-Robertson pupils
3	±	0	0	0	0	0	0	0(0)0	25	Paresthesias; peroneal palsy
4	±	0	0	0	0	0	0	0	25	None
5	±	0	0	0	0	0	0	0	25-50	Eighth nerve deafness?
6	+	0	0	0	0	0	0	0	25-50	History not available
7	+	0	0	0	0	0	0	0	25	Pupillary changes
8	+	0	0	0	0	0	0	000000	25	General paresis, incipient
9	+	+±	0	0	0	0	0	110000	25	None
10	+	+	0	0	0	0	0	0	25	None
11	+	+	±	0	0	0	0	0	25	General paresis, incipient
12	+	+	+	0	0	0	0	0	25	Seventh nerve palsy
13	+	+	+	0	0	0	0	0	50	History not available
14	+	+	+	±	0	0	0	0	50	None
15	+	+	+	+	0	0	±	111000	25-50	Pupillary changes; paresthesia; ulnar hyperesthesia
16	+	+	+	+	0	0	0	0	25	Mental deterioration
17	+	+	+	+	0	0	0	0	25	Tabes
18	+	+	+	+	0	0	+	221000	50	Taboparesis
19	+	+	+	+	0	0	Not done	Not done	Not done	Seventh and eighth nerve palsy
20	+	+	+	+	0	0	++	111000	50	Eighth nerve deafness; paresthesias
21	+	+	+	+	±	0	0	0	25	Third and seventh nerve palsy; pupillary changes; optic neuritis?
22	+	+	+	+	±	0	0	0	25	Argyll-Robertson pupils
23	+	+	+	+	+	0	0	0	25	Tabes
24	+	+	+	+	+	0	±	222000	25	Tabes
25	+	+	+	+	+	±	+	554300	75	None
26	+	+	+	+	+	+	++	444211	50	None
27	+	+	+	+	+	±	0	0	25	Optic atrophy—Argyll-Robertson pupils
28	+	+	+	+	+	+	0	022222	50	Tabes
29	+	+	+	+	+	+	+	332100	50	General paresis, incipient

TABLE III A

PROTEIN WITHIN NORMAL LIMITS; PANDY AND MASTIC TESTS NEGATIVE	VERY SLIGHT INCREASE IN PROTEIN PANDY AND MASTIC TESTS DOUBTFUL	DEFINITE INCREASE IN SPINAL FLUID GLOBULIN
18	4	6

22 out of 28 fluids with a positive Wassermann reaction and a negative precipitation test yield no significant precipitate upon half-saturation with 50 per cent $(NH_4)_2SO_4$

taining small amounts of reagin, say 1, 2, 4, or even 8 units, this serum inhibitor may prevent complement fixation entirely, while the precipitation reaction, although less delicate than complement fixation, will, since it is relatively unaffected by the inhibitor, be positive. Nor can one escape the inhibition in the Wassermann reaction by using dilute serum: for obviously, if one uses 1:5 serum instead of whole serum in the Wassermann reaction, there is not only less inhibitor, but also less reagin, and the net result is a further loss in sensitivity.

The greater sensitivity of the precipitation test with serum as compared with the Wassermann reaction is thus explained, for only those sera which contain enough reagin to overcome the serum inhibition will give a positive Wassermann reaction. In spinal fluids, however, which normally contain less than 25 mg. per cent of protein, that is, an amount less than 1/200 the serum protein concentration, and which even in general paralysis rarely contain more than 400 mg. per cent (less than 1/15 the serum protein concentration), this inhibiting substance is not present, as is shown experimentally in Table IV. In the absence of the inhibitor,

TABLE IV

SHOWING THAT SPINAL FLUID CONTAINS LITTLE OR NO INHIBITING SUBSTANCE FOR COMPLEMENT FIXATION

DILUENT	WASSERMANN RESULT OF A SYPHILITIC SERUM DILUTED								
	1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
NaCl 0.85 per cent	+	+	+	+	+	+	+	+	0
Normal inactivated serum	+	+	+	+	±	0	0	0	0
Normal spinal fluid	+	+	+	+	+	+	+	±	0

the intrinsically greater delicacy of the fixation phenomenon becomes immediately apparent in the results obtained by the spinal fluid Wassermann reaction in comparison with the precipitation test (Table I).

SUMMARY

A sensitive ice box Wassermann reaction is intrinsically several times as delicate as the precipitation tests in current use. Serum, however, contains an inhibiting substance or factor, associated with the albumin fraction, which prevents complement fixation without appreciably affecting precipitation, thus causing the latter to be a considerably more sensitive test for small quantities of serum reagin than the Wassermann reaction.

Spinal fluid apparently does not contain this inhibiting factor, probably because its protein content is normally only 1/300 that of serum. The intrinsically greater delicacy of complement fixation becomes immediately apparent, the Wassermann reaction detecting 40 to 50 per cent more positive spinal fluids than the precipitation test, despite the fact that the latter detects 20 to 30 per cent more syphilitic sera.

The Kahn procedure of attempting to increase the sensitivity of the precipita-

tion tests with spinal fluid by concentrating the globulin with $(NH_4)_2SO_4$, was found to be quite unsatisfactory, for reasons described in the text. Some improvement in the sensitivity of the author's spinal fluid precipitation test was achieved by decreasing the antigen: spinal fluid ratio to 1:200, as compared with 1:10 for serum. Nevertheless, the Wassermann reaction remains the method of choice in the examination of spinal fluids; so long as this holds true, it cannot be entirely supplanted by any precipitation test, no matter how great the superiority of the latter with serum.

REFERENCES

1. Eagle, H.: Studies in the Serology of Syphilis. VIII. A New Flocculation Test for the Serum Diagnosis of Syphilis, J. Lab. & Clin. Med. **17**: 787-791, 1932.
2. Eagle, H.: Studies in the Serology of Syphilis. XI. A Clinical and Statistical Evaluation of a New Precipitation Test for Syphilis, J. Lab. & Clin. Med. **18**: 684, 1933.
3. Kahn, R. L.: The Laboratory Diagnosis of Syphilis by Precipitation, Williams and Wilkins, Baltimore, 1923.
4. Noguchi, H., and Bronfenbrenner, J.: The Interference of Inactive Serum and Egg White in the Phenomenon of Complement Fixation, J. Exper. Med. **13**: 92-97, 1911.
5. Sellards, A. W., and Minot, G. R.: The Antagonistic Action of Negative Sera Upon the Wassermann Reaction, J. M. Research **34**: 131-147, 1916.
6. Walker, R. M.: On the Colloidal Nature of the Wassermann Reaction, J. Path. Bact. **21**: 184-192, 1917.
7. Ronchese, A. D.: Quelques Caractères differenciant la reaction de Bordet—Wassermann des reactions de fixation proprement dites, Compt. rend. Soc. de Biol. **81**: 837-9, 1918.
8. Eagle, H.: Studies in the Serology of Syphilis. V. The Cause of the Greater Sensitivity of the Ice Box Wassermann, J. Exper. Med. **53**: 615-623, 1931.

NEW DETAILS OF TECHNIC IN AIR ANALYSIS*

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THE indirect heat measurement and the respiratory quotient, derived from the analysis of air, have come into such common use that any factors affecting the accuracy of these measurements are of interest. The observations recorded below are a result of several years of work with an apparatus designed and described by Carpenter[1] and also with a similar one as modified by Du Vigneaud.[2] The latter apparatus, described by Du Vigneaud,[2] is adapted for the analysis of expired air as well as outdoor air. A small bulb on the burette separates the range of CO_2 percentages of outdoor air from those of expired air. The mercury trap was found to be very practical in keeping the burette clean. The mercury and the KOH leveling bulbs were fitted with rack and pinion adjustments and a new potassium pyrogallate bulb of about 600 c.c. capacity replaced the original one.

The purpose of this paper is not to repeat the excellent directions and precautions already stated by Carpenter,[1, 3, 6] but rather to point out the application and importance of new details heretofore unpublished. I am indebted to Dr. T. M. Carpenter for frequent helpful discussion. The results set forth in this paper are limited strictly to new considerations and to points on which, over a period of

*From the Institute of Animal Nutrition, Pennsylvania State College.
Received for publication, June 21, 1932.

years, consistent results require a viewpoint and interpretation somewhat different from those of Carpenter. The analyst must of course be his own judge as to the correctness and importance of these considerations.

SIMULTANEOUS ABSORPTION OF OXYGEN AND CARBON DIOXIDE

Since the accuracy of the respiratory quotient value depends in part on the magnitude of the percentages of CO_2 produced and oxygen consumed, there is need for greater accuracy in analysis when respiration chambers are used than when undiluted expired air is analyzed. In the usual method of analysis, an error in the CO_2 determination causes an error in the corresponding oxygen determination; though if the initial and final volumes are correct, these errors tend to compensate each other as they affect the respiratory quotient. The following "Total absorption" method ($CO_2 + O_2$) is short and accurate, and is particularly adaptable to samples taken from respiration chambers, in which the percentage of CO_2 is relatively low, or under any conditions which involve an extensive number of samples.

The usual method followed in air analysis is time-consuming chiefly because the air in the capillary tube above the KOH must be freed of oxygen by a process of dilution with nitrogen. In the routine pertaining to oxygen absorption as outlined by Carpenter,[1] for instance, half of the number of trips of the sample into the pyrogallol are required for this reason, and not because of any lack of efficiency in the absorption of oxygen by the pyro.

The modification in method consists of putting the sample directly into the pyro, which, of course absorbs both the oxygen and CO_2. Ten trips of the sample into the pyro, which require about seven minutes, result in complete absorption of these gases. The levels are set as usual, with the sample open to the KOH.

Before again putting the sample into the pyro (5 times), to check the completeness of absorption, the sample is put once into the KOH to sweep out any oxygen which might have diffused beyond the stopcock above the KOH while the initial volume was being recorded. Concordant readings, taken after 10 trips and after 15 trips as described testify, however, that no such extensive diffusion takes place during the time required for careful adjustment of the KOH levels when measuring the original sample.

This method of obtaining the total absorption ($CO_2 + O_2$) is very short and direct, and minimizes any effects of changes in temperature or barometer during the course of an analysis. Excellent results have been obtained on many scores of samples by this method.

The total absorption value may be obtained independently of any values subsequently obtained for CO_2. Thus, the average value of three total absorptions, combined with the average of three CO_2 determinations, gives better data for the determination of oxygen, and a respiratory quotient, than do three analyses done in the usual manner. The fact that twice as large a volume of sample is required by the method of determining the CO_2 and total absorption separately does not detract from its usefulness since the amount of air available for analysis is not ordinarily limited.

If the CO_2 is measured gravimetrically, only the total absorption value need be determined. Though the apparatus, following the determination of total absorption, may be used at once for CO_2 determinations, it must be freed of oxygen

before the order is reversed. The use of one apparatus for CO_2 determinations and another for total absorption makes this unnecessary.

THE EFFICIENCY AND CAPACITY OF PYRO SOLUTION AS AN OXYGEN ABSORBENT

In the preparation of a pyrogallate solution it is obvious that contact with the air should be avoided. This simple precaution has formerly been apparently overlooked. Carpenter states[1] that one filling of the apparatus is sufficient for only 10 analyses but mentions the use of "a long stem glass funnel" in introducing the pyro into the apparatus. The rapidity with which a pyro solution absorbs oxygen has been observed in the course of many routine analyses. After the sample has been forced into the pyro 3 times, more than 99 per cent of the oxygen is already absorbed. This fact, quickly demonstrable with any apparatus, shows the pyro to be an extremely efficient absorbent, especially since the whole sample is never exposed to the pyro at any one time. This rapidity of absorption has not previously been properly evaluated. A proper consideration of the speed and completeness of absorption of oxygen by the pyro when used in a small[1] or large[2] bulb makes it clear that any modification of the form of the bulb or any circulation of the pyro[5] can accomplish but little. Satisfactory mixing of the pyro solution obtains as a result of passing the sample into and out of it. This is indicated by the continued efficiency of the pyro solution. It is also shown by the rapidity and completeness of thorough mixing when two solutions of different colors are placed in opposite sides of the potassium pyrogallate pipette and an air sample forced over in the usual manner. There is therefore in the absence of a circulating device no likelihood that "fresh unused solution"[5] is a factor of any importance. The modified form of the potassium pyrogallate pipette[5] has the alleged purpose of furnishing fresh pyro to the air sample, but no claim is made that any fewer trips of the sample into the pyro are required. Therefore, no time is saved. The larger bulb in the modified form[5] is useful because "75 analyses may be carried out without renewal of the pyrogallate solution." By actual count the writer performed 483 analyses with a similar amount of pyro, checking the analyses from time to time with samples of outdoor air. The pyro seemed as efficient during the last analysis as during the first. The pyro solution was prepared and introduced into the apparatus in the manner described below. Seventy-five or more analyses (instead of ten) may be made from one filling of the small bulb of the earlier form of the apparatus.[1] This is true only if care is taken that the pyro is not largely exhausted by exposure to room air before being actually used for air analysis. One filling of the apparatus may be insufficient even for 10 analyses if the pyro is poured from one container to another in the open air.

In preparing a pyrogallate solution, the practice in this laboratory has been to add a weighed amount of pyrogallic acid to a known amount of strong KOH solution,* previously placed in an eight-liter bottle, and immediately to stopper the mouth with a 2-holed stopper fitted with 2 glass tubes which are closed by rubber connections and clamps. (A little mineral oil may be placed on the surface if care be taken that no oil later is allowed to reach the inside of the air analysis apparatus.)

*In the ratio of 10 grams of pyrogallic acid dissolved in 100 c.c. of KOH solution having a specific gravity of 1.55 at 20° C.

To avoid contact of the pyro solution with oxygen in the process of filling the absorption pipette, connect one of the glass tubes in the stopper of the supply bottle with the bottom of the pipette, and the other with a small bottle of pyro. Then elevate the supply bottle so that the pyro runs through one tube into the pipette, while air is drawn into the supply bottle through the other tube and the small bottle of pyro.

As a test of the efficiency and capacity of pyro to absorb oxygen, dry CO_2-free air was drawn through known quantities of fresh pyro solution placed in two absorption bottles connected in series. The rate of air flow was about 225 c.c. per minute. The bottles of pyro and the sulphuric acid which followed each were weighed from time to time until no further increase in weight occurred. Three tests showed that fresh pyro can absorb about 15.5 c.c. of oxygen per gram of pyro. Under these conditions the first bottle of pyro let a small amount of oxygen through to the second, even at the beginning, but this amount did not increase at any greater rate until after the first solution had become 65 per cent exhausted.

The conditions of this test were much more severe than those encountered in air analysis, in that the oxygen had only one momentary contact with the pyro. (It may also be pointed out that the maximum quantity of oxygen absorbed in this test undoubtedly exceeds the amount which the pyro will absorb under the conditions of analysis when the last traces must be quantitatively removed.)

Since the efficiency of the pyro is not appreciably diminished until ⅔ exhausted, 100 c.c. of fresh solution for air analysis should quantitatively absorb about 1800 c.c. of oxygen. Seventy-five cubic centimeters of pyro in an apparatus having a measuring burette of 40 c.c. capacity,[1] therefore, should be sufficient for at least 150 analyses.

In my practice the number of analyses has never exceeded 80, though it was never demonstrated that the efficiency of the pyro solution was impaired. The inconvenience of introducing a new solution of pyro into the apparatus is considerable. A larger bulb which permits a proportionately larger number of analyses thus serves a very useful purpose.

THE ABSORPTION OF WATER BY THE PYROGALLIC ACID SOLUTION

When the sample volume is recorded by reading the height of the mercury in the calibrated burette, the amount of water on the mercury must be subtracted from the apparent volume of the sample. Due to the dehydrating action of the pyro, however, an error is involved if the same amount of water is used in correcting both the initial and final volumes of the sample. The dehydrating effect of the pyro solution has been recognized by others but so far as I am aware no attempt has been reported to evaluate this dehydrating action of the pyro in the computation of the oxygen percentage.

To measure the amount of water removed from the sample by the pyro solution an appropriate volume of sample (nitrogen), about 79 per cent of the total volume of the burette, was forced into the pyro solution 150 times, in exactly the same way as during the determination of total absorption. There was removed by this procedure 0.102 per cent of water as read on the burette, or 0.010 per cent per 15 trips, the number required in the determination of total absorption. The sample was put back and forth into the pyro without any appreciable pause between trips,

thus duplicating the routine ordinarily followed. In addition to this special test the amount of water taken up by the pyro has been verified many times during years of work with this type of apparatus. The length of time that the pyro has been in the apparatus, exposed to the water seal, and the number of oxygen determinations for which it has been used, may possibly affect its dehydrating action, though any such influence has been too small to detect. A small amount of water is used in saturating the initial sample and varies with temperature, but this amount is negligible, especially since no sample was wholly dry when introduced into the apparatus. (The amount of water necessary to saturate a dry sample (40 c.c.) at 23° C. is only about 0.002 per cent as read on the Carpenter burette.)

It is therefore apparent that the volume of water subtracted from the final reading should be 0.010 per cent smaller than that used to correct the original volume. This is an important consideration since this difference is reflected nearly quantitatively in the computed oxygen or total absorption percentage. If the amount of water read, after ample time for complete drainage, is applied to the initial volume of the following sample, the next few water values can be accurately estimated without actually reading the water, checking up the last estimation with the observed amount whenever it is convenient to allow time for complete drainage.

It might be objected that a change in temperature may affect the amount of water condensed. The volume of the sample at the end of an analysis is about 30 c.c. If the room temperature should drop 6° C., say from 23° C. to 17° C., the amount of water condensed would be increased by only 0.00018 c.c. or about 0.0005 per cent as read on the burette!

Any water above the mercury, whether directly on the mercury or clinging to the sides of the burette, occupies volume, and if ignored is measured as gas. In the foregoing test the burette was thoroughly clean, a condition necessary to obtain good analyses, and particularly imperative in this particular test.

The following figures illustrate the methods of computation. The foregoing discussion indicates that the percentage of oxygen (or of total absorption) should be computed as indicated in column B. Column A shows the ordinary method.

TABLE I

	A	B
Initial reading	99.965	99.965
Volume of water	0.186	0.196
Volume of sample	99.779	99.769
Volume after CO_2 absorption	99.935	99.935
Volume of water	0.186	0.196
Volume of sample	99.749	99.739
Volume after O_2 absorption	79.041	70.041
Volume of water	0.186	0.186
Volume of sample	78.855	78.855
Per cent oxygen	20.940	20.932

THE ABSORPTION OF OXYGEN AND NITROGEN BY THE KOH SOLUTION

A steady increase in the volume of gas measured (starting with nitrogen) was noted as a result of repeated trips of the sample into the KOH solution. These trips were made in the shortest time practicable, single readings only being taken. Any slight irregularity in the increase in volume is therefore not significant, but

since errors of individual readings are not additive, the general situation prevailing is clearly shown. (Table II.) The first reading in colum B follows the last reading in column A, etc.

After the last traces of O_2 have been removed from the sample, the KOH is then exposed to pure nitrogen on one side and to room air, or about 79 per cent nitrogen, on the other. Many trips of the nitrogen into the KOH under these conditions naturally tend toward equilibrium; or, in other words, toward a loss of nitrogen from the apparatus to the room air, through the KOH. This slight loss is shown by the decrease in the first values listed in columns A, B, C, and D. However, a larger change in the opposite direction takes place in the case of O_2, due to its greater solubility, and to the conditions of partial pressures which prevail. This increase is shown in the columns of Table II. After the last traces of O_2 have been removed from the sample, the KOH is then exposed to pure nitrogen on one side and to room air, or about 21 per cent O_2, on the other. Many trips of the sample of nitrogen into the KOH under these conditions naturally tend toward equilibrium, or in other words, toward a gain of O_2 from the room air, as well as a loss of nitrogen to the same. This gain of O_2 is much greater than the loss of nitrogen, as indicated by the data in Table II. Each reading, in fact, represents the algebraic sum of the nitrogen lost and the oxygen gained. After the sample has increased 0.06 or 0.07, as a result of 35 trips to the KOH, and is then put into the pyro 10 times, to remove the oxygen, the remaining nitrogen is slightly less than before the series of trips was made.

TABLE II

DATA SHOWING THE LOSS AND ABSORPTION OF O_2 AND N_2 FROM A 10 PER CENT KOH SOLUTION

	A	B	C	D
Direct from pyro (10 trips) read at once	79.156	79.138	79.124	79.103
Direct from pyro after one minute	79.159	79.141	79.126	79.105
Direct from pyro after five minutes	79.162	79.143	79.128	
After 1 trip to KOH	79.170	79.148	79.131	
After 2 trips to KOH	79.170	79.148	79.131	
After 5 trips to KOH	79.177	79.159	79.136	
After 10 trips to KOH	79.183	79.173	79.141	
After 15 trips to KOH	79.191	79.173	79.157	
After 20 trips to KOH	79.201	79.186	79.154	
After 25 trips to KOH	79.209	79.189	79.163	
After 30 trips to KOH	79.218	79.195	79.169	
After 35 trips to KOH	79.223	79.200	79.174	

A further indication that the KOH solution absorbs or loses O_2 and N_2, under the conditions stated above, is that no such change in volume takes place during the regular determinations of CO_2 when the air on both sides of the KOH contains almost exactly the same percentages of O_2 and N_2.

As a further check on the situation indicated by the above data, nitrogen was put on both sides of the KOH. The compensating bulb was also filled with nitrogen. By opening a screw cock to the room, when the levels were set, this arrangement did not interfere with the sensitivity or with the compensation.

A routine similar to that followed before showed a temporary increase in volume as the nitrogen was put into the KOH, but this soon ceased altogether. After a few trips into the KOH, and into the pyro, to remove oxygen, a series of 50

trips gave no change in volume. The small initial increase is due to oxygen in solution slowly given up to an atmosphere of pure nitrogen.

Leaving the nitrogen in the compensator and on the external side of the KOH, an outdoor air sample was taken in for CO_2 analysis. Readings were taken after 6, 10, 20, 30, 40, and 50 trips of the sample into the KOH. The readings indicated the following percentages of CO_2: 0.047, 0.051, 0.061, 0.068, 0.075, 0.090. This indicates again that the KOH absorbs oxygen under these conditions.

After the nitrogen was removed from the external side of the KOH, and from the compensator, the sample which contained about 21 per cent oxygen was driven into the KOH many times. Then a series of 50 trips resulted in no change in volume.

The practical point in regard to the situation is that since the introduction of nitrogen into the KOH involves a possible error, I believe that the procedure is undesirable and unnecessary. If the sample is taken back slowly from its last trip to the pyro, and a little additional time allowed to complete the saturation (as when taking the initial volume) the error can be minimized.

Since the KOH solution at the end of an analysis is exposed to pure N_2 on one side, and to room air on the other, there is a chance of a slight error, even though the sample is not actually forced into the KOH. To have the KOH protected from the room air by a bulb of N_2 would be ideal for the determination of the total absorption. The conditions which prevail in the usual set-up are nearly perfect for the determination of CO_2. The simplest procedure seems to be the use of one apparatus for the determination of CO_2, and another (with the KOH replaced by water) for the determination of total absorption. The one used for total absorption would have N_2 on each side of the KOH. As indicated above, this is desirable only when a considerable number of samples are at hand.

It should be noted that by ignoring the change in the amount of water on the mercury during an analysis the resulting oxygen value is too high. Putting the sample of nitrogen into the KOH solution at the end of an analysis gives an oxygen percentage which is too low. These two errors, being of the same general magnitude nearly compensate. This fact, however, hardly justifies ignoring them.

THE COMPOSITION AND STORAGE OF OUTDOOR AIR

The following results were obtained on outdoor air by determining both the total absorption and the CO_2, in duplicate, or in triplicate. These data show the extent of uniformity found in the composition of outdoor air.

TABLE III

DATE	TOTAL ABSORPTION	CO_2	O_2
March 25	20.979	0.036	20.943
March 26	20.977	0.035	20.942
March 29	20.968	0.029	20.939
April 2	20.968	0.030	20.938
April 22	20.974	0.034	20.940
May 4	20.973	0.034	20.939
May 11	20.973	0.035	20.938
May 18	20.974	0.033	20.941
Average	20.973	0.033	20.940

The analysis of outdoor air, therefore, and the determination of CO_2 and O_2 (and a respiratory quotient) from burning acetone,[3] serve well in checking the correctness of results of air analysis.

The use of a satisfactory respiratory quotient as a sole criterion in testing the tightness of a metabolism apparatus is inadequate since a leak of room air into it reduces both the apparent CO_2 production and oxygen consumption, and thus may scarcely at all affect the respiratory quotient.

Samples of outdoor air were taken and stored in glass sampling tubes and analyzed after one week and two weeks to determine the change in composition during storage. These samples contained the water vapor of outside air at the time of sampling. One group (A) was stored in perfectly clean tubes, another (B) in tubes covered on the inside surface with a film of mercury, and a third group (C) contained an appreciable quantity of mercury in the tubes. As stated by Haldane[4] the tubes should be dry and clean. The results are shown in Table IV below, and require no special comment except to point out that the oxygen percentage decreased in the tubes in which samples were stored with even small amounts of mercury.

TABLE IV

DAYS OF STORAGE	A		B		C	
	O_2	CO_2	O_2	CO_2	O_2	CO_2
0	20.939	0.034	20.939	0.034	20.939	0.034
7	20.940	0.035	20.940	0.035	20.920	0.035
14	20.941	0.033	20.922	0.033	20.894	0.029

SUMMARY

In the analysis of air, it is desirable to determine the CO_2 and O_2 together, separate from the determination of CO_2, if many samples are to be analyzed.

The protection of the pyro solution from exposure to the air, during introduction into the absorption pipette, materially extends the usefulness of the solution.

To disregard the dehydrating action of the pyro solution causes a plus error in the computed oxygen percentage.

Forcing the sample of nitrogen into the KOH solution, at the completion of an analysis, causes a minus error in the computed oxygen percentage.

Samples of outdoor air stored in tubes containing mercury show a decrease in oxygen.

REFERENCES

1. Carpenter, T. M.: An Apparatus for the Exact Analysis of Air in Metabolism Investigations With Respiratory Exchange Chambers, J. Metab. Res. **4**: 1, 1923.
2. Du Vigneaud, V.: Some Useful Modifications of the Haldane Gas-Analysis Apparatus, J. LAB. & CLIN. MED. **13**: 175, 1927.
3. Carpenter, T. M., Fox, E. L., and Sereque, A. F.: Acetone as a Control Substance for Respiration and Gas Analysis Apparatus, J. Biol. Chem. **82**: 335, 1929.
4. Haldane, J. S.: Methods of Air Analysis, Philadelphia, 1912, J. B. Lippincott Co.

5. Carpenter, T. M., Fox, E. L., and Sereque, A. F.: The Carpenter Form of the Haldane Gas Analysis Apparatus. Changes Made in the Apparatus and Details Regarding Its Use, J. Biol. Chem. **83**: 211, 1929.
6. Carpenter, T. M., Lee, R. C., and Finnerty, A. E.: Ein Apparat für die exakte und schnelle Analyse der Gase aus einer Respirationskammer, Wissenschaftliches Arch. f. Landwirtschaft **4**: 1, 1930.

AN AUTOMATIC METHOD OF RECORDING BLOOD FLOW*

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FOLLOWING the discovery of the circulation of blood by Harvey in 1615 many methods have been devised to measure the rate of blood flow. Since the activities (at least in a quantitative sense) of many organs are revealed only by a study of the blood, knowledge of the quantity of blood flowing through an organ is of fundamental importance.

In the main there have been two principal types of apparatus used. One, various modifications of the stromuhr first devised by Ludwig and two, thermoelectric stromuhrs. The older methods are described in Tigerstedt's *Handbuch der Physiologischen Methodik*. Of the modifications of the Ludwig stromuhr, two should be mentioned. Barcroft (1929) built a mechanical stromuhr which appears to be an excellent instrument. More than ordinary mechanical skill, however, is required in fabricating the instrument. Montgomery and Lipscomb (1929) constructed an instrument in which the blood causes a column of mercury to be displaced, which in turn activates a Pavlov electromagnetic valve (1887). It is a satisfactory means of measuring arterial flow but the back pressure developed would certainly limit its usefulness on the venous side of an organ. Gesell and Bronk (1926), Rein (1928), and Herrick and Baldes (1931) have used the principle of the second group, the thermoelectric stromuhr. This method certainly is the most physiologic because the electrodes and thermocouples are approximated to the blood vessel in situ without severing the vessel. However, the method does not measure the flow with great accuracy. Herrick and Baldes after an extensive investigation found it to be reliable only within 10 per cent.

A method has been described by Gayet and Guillaumie (1930). The blood flow is interrupted from its regular course and directed into a measuring chamber of known volume. The time necessary to fill the chamber is noted. Then the original circulation is made and the blood drawn permitted to flow into the animal. The method, we believe, is satisfactory for approximate work but the human error is difficult to evaluate and the method does not give a continuous measurement.

We have investigated various mechanical stromuhrs and have built one in which many of the objectionable features of others are minimized or eliminated.

DESCRIPTION OF THE STROMUHR

Fig. 1 shows structure and dimensions of the glass stromuhr. Fig. 2 shows the construction of the Pavlov valve and the stromuhr with tubes in place. Fig. 3 is the wiring diagram of the apparatus which operates the valve.

*From the Department of Physiology, University of Chicago.
Received for publication, July 14, 1932.

OPERATION

The four platinum electrodes are heavily electroplated with copper. The Y tubes which are connected to the inlet-outlet of the stromuhr are crossed so that when the bar of the valve is forward the blood will enter one side and leave by the other. When the bar is pulled back the direction of flow in the Stromuhr is reversed. The balls of the stromuhr are coated inside with a paraffin-wax mixture. The tube "A" (Fig. 1), which connects the two cylinders "B" is filled with chloroform just to the level of the electrodes by way of the stopcocks at the top of the stromuhr. An additional amount is then added equal to the desired volume transfer of blood per shift of the valve. A saturated copper sulphate solution is placed in both cylinders "B" to a point about halfway between the upper electrode and

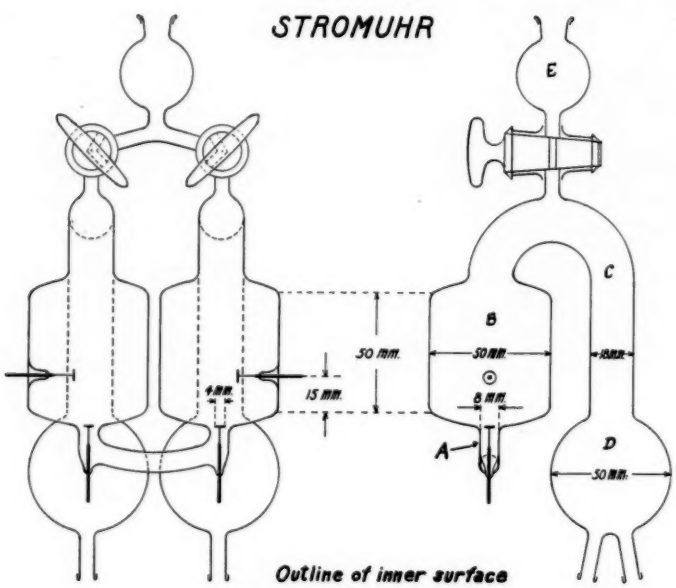


Fig. 1.

the top of the cylinder. Now place the inlet and outlet tubes from the balls "D" in a beaker of saline and by gentle suction at "E" half fill them with the saline. Then clamp the inlet-outlet tubes. Fill the reservoir "E" with a high grade mineral oil of low viscosity (L. G. White Mineral Oil sp. gr. 847, viscosity 75-80, Standard Oil Company of Indiana). By intermittent *gentle* suction one can fill the remaining space of "B," "C," and "D" with the oil. Then close the stopcocks. Connect the inlet tube to a reservoir containing saline and the outlet to a measuring flask, and close the switches which connect the stromuhr electrodes with the relays. The apparatus is now ready to be calibrated. Permit fluid to pass through the stromuhr at about the rate of blood flow to be measured until the valve has shifted about 50 times. The volume measured divided by the number of shifts is the shift-volume, the magnitude of which will depend upon the volume of the $CHCl_3$ used in the apparatus. There is a small error introduced when the rate of

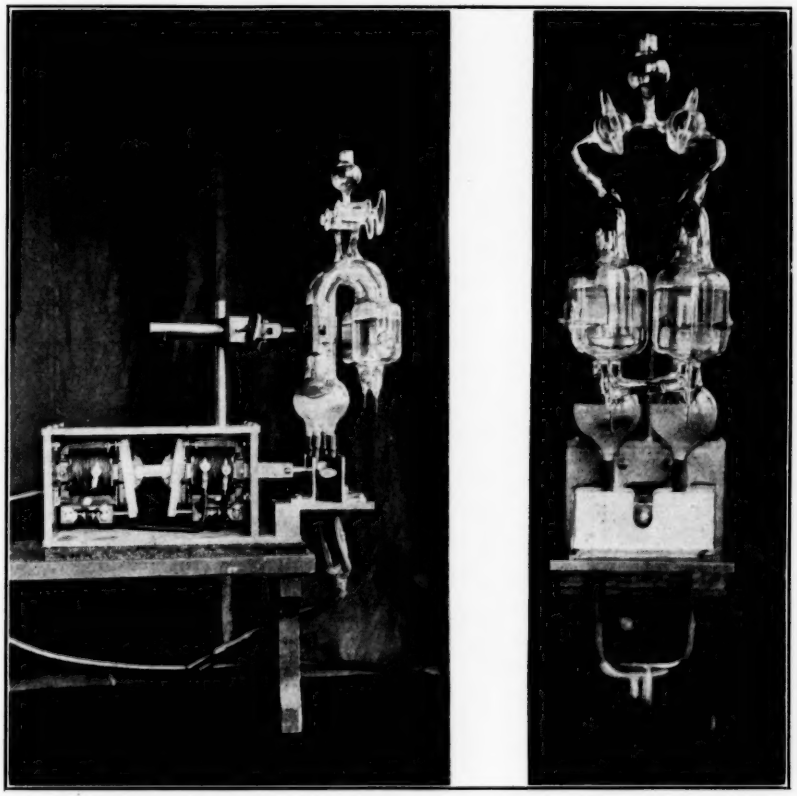
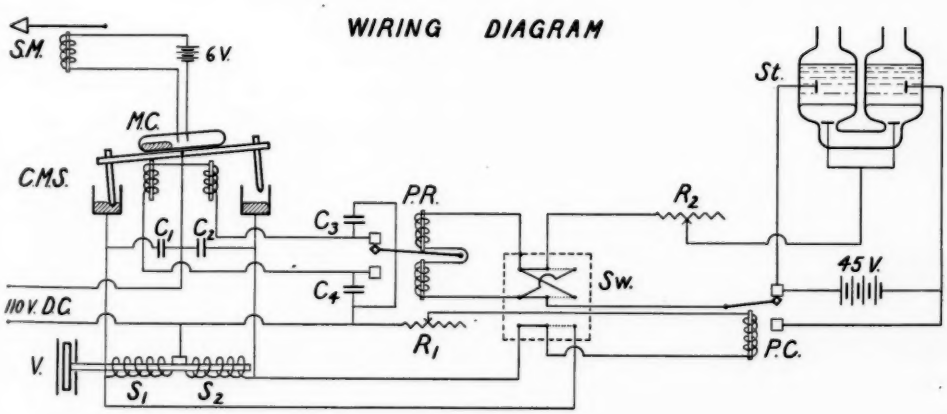


Fig. 2.



C_{1-4} 2 mf. condensers
C.M.S. carbon-mercury switch
M.C. mercury contact
P.C. 400 ohm pole changer W.U.T.Co.
P.R. 600 ohm polar relay 6-c W.U.T.Co.
R_1 R_2 3000 ohm rheostats
S_1 push solenoid CR-9503-209 c G.E.Co.
S_2 pull solenoid CR-9503-209 G.E.Co.
S.M. signal magnet
Sw. triple pole double throw switch
St. stromuhr
V. valve

Fig. 3.

flow changes. Fig. 4 shows the error to be less than 1 per cent when the rate of flow is increased 100 per cent over the rate used for calibration.

Although the tendency of the electrodes to gas is minimal the use of the pole change (or relay) P. C. eliminates that objectionable feature. In Fig. 3 it will be noted that as soon as relay P. R. closes to operate C. M. S. and S., that P. C. opens the circuit between the functioning electrodes and connects the battery (with reversed polarity) to the other pair of stromuhr electrodes. Thus the current flows between the electrode only momentarily. Switch S W is used to reverse the polarity of the battery to the stromuhr and prevent deplating the electrode.

We have used heparinized animals in our work. One full dose of heparin (15 mg. per kilo) is given intravenously just before connecting the stromuhr to the animal. One dose per hour is then administered by a constant injection apparatus.

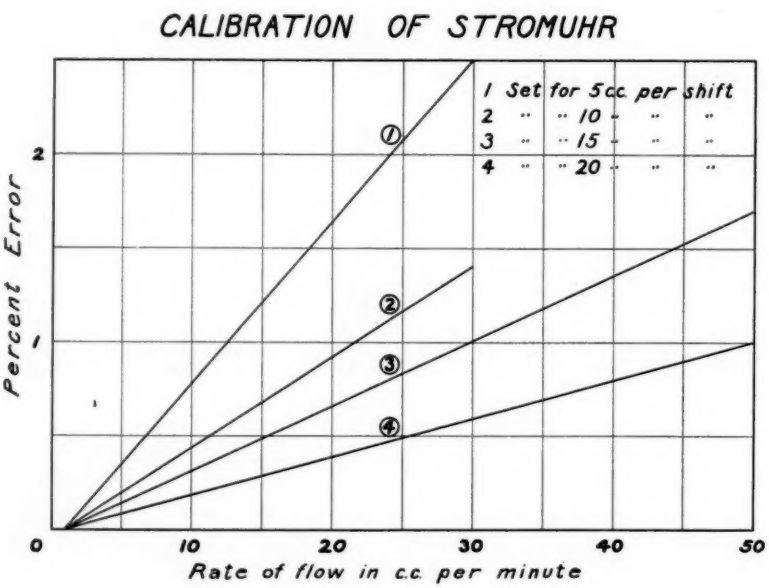


Fig. 4.

The tubes leading from the animal to the stromuhr should be as short as possible. We place the valve and stromuhr on a heavy bracket just above the animal.

This apparatus has been used by us to measure blood flow on the venous side of organs with very satisfactory results. The parts are neither expensive nor difficult to build. It is entirely automatic in operation and the back pressure is less than 7 mm. of water.

ADDENDUM

We now use two relays (Struthers Dunn Inc., Philadelphia, type ABYS8) in place of the carbon mercury switch (C.M.S.). The resulting decreased lag reduces the errors charted in Fig. 4 to one-third the values given.

We wish to express our appreciation to Messrs. Rust and Clark of the Western Union Telegraph Company of Chicago for the gift of the relays used in this work.

REFERENCES

Barcroft, H.: A Mechanical Stromuhr, J. Physiol. **67**: 402, 1929.
Burton-Opitz, R.: Ein Stromuhr fur die Messung der Blutvolumina der Venen, Arch. f. d. ges. Physiol. 121-150, 1908.
Gayet, R., and Guillaumie, M.: Présentation d'un hémorhéomètre constitue avec des segments vasculaires, Compt. rend. soc. de biol. 103-1102, 1930.
Gesell, R.: On the Relation of Blood Volume to Tissue Nutrition; Automatic and Bloodless Method of Recording Volume Flow of Blood, Am. J. Physiol. **70**: 254, 1924.
Gesell, R., and Bronk, D. W.: The Regulation of Respiration, Am. J. Physiol. **79**: 61, 1926.
Herrick, J. F., and Baldes, E. J.: The Thermo-Stromuhr Method of Measuring Blood Flow, Physics **1**: 407, 1931.
Montgomery, M. T., and Lipscomb, T. H.: Am. J. Physiol. **90**: 454, 1929.
Rein, H.: Die Thermo-Stromuhr, Ztschr. f. Biol. **87**: 394, 1928.

A STUDY OF VITALI'S TEST FOR ATROPINE*

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VITALI'S test is used to characterize the alkaloids, veratrine and atropine. With this test the former gives an orange to reddish violet coloration, whereas the latter gives a deep reddish violet color. The reaction depends upon the oxidation of atropine with concentrated nitric acid and the subsequent formation of an intense reddish violet color† on the addition of alcoholic potassium hydroxide solution. The color after considerable time changes to a cherry-red.

The test was proposed in 1880 by Vitali[1] who tested over 60 alkaloids and found that only 3, daturine, hyoscyamine, and duboisine, gave the violet color.

Witthaus[2] reports that in addition to hyoscyamine, scopolamine gives the test. Van Urk[3] found several alkaloids and other substances which gave similar reactions. Celsi[4] has reported that the positive test is given by the esters of aromatic acids but not by the free acid.

Fuller[5] in dealing with crude drugs found the petroleum ether extract from belladonna, coca, nux vomica, and yohimbe to give a color reaction similar to atropine.

A study of Mandelin's and Fröhde's tests for alkaloids in this laboratory[6, 7] has shown that these tests are not specific. Levine[8] and Levine and Magiera[9] have found certain alkaloidal reagents to be more or less general reagents for phenols.

The object of the investigation reported in this paper was to test a large number of organic compounds using Vitali's test, in order to determine whether or not the characteristic violet color was given by any number of the organic compounds. Also, to determine whether or not this coloration was due to any particular organic grouping. The effect of impurities on the test was also studied.

PROCEDURE

Preparation of Standard Atropine Solution.—A solution was prepared in alcohol containing one milligram of atropine per cubic centimeter.

Preparation of Solutions to be Tested.—The compounds to be tested were dis-

*From the Department of Chemistry, University of Colorado.
Received for publication, July 11, 1932.
†Most textbooks describe the color as violet.

solved in the proper solvent so that each cubic centimeter was equivalent to one milligram of the compound.

Procedure of Tests.—Three tests were made on each compound. For the first test, one cubic centimeter of the solution to be tested was evaporated to dryness, two or three drops of fuming HNO_3 were added, and again evaporated on a water-bath. After cooling, three drops of alcohol KOH were added and any coloration noted. In the second test, one cubic centimeter each of the organic compound and standard atropine solutions were mixed and the test made as described above. If there were no interference, a deep violet color should result. The third test was carried out as above, except that five cubic centimeters of the solution of the organic compound were used with one cubic centimeter of the atropine.

The results obtained when Vitali's test was applied to the organic compounds are given in the pages immediately following. The name of the organic compound is given first, and directly opposite is given the color reaction. In cases where no color was developed other than that caused by the reagents, the compounds are listed with the statement, "No color reaction."

ALKALOIDS AND ALKALOIDAL SALTS

ORGANIC SUBSTANCES	COLOR REACTION
Apomorphine	Greyish brown
Atropine	Deep reddish violet
Belladonnine	Deep reddish violet
Berberine	Greyish brown
Cinchonine	Orange
Codeine	Yellowish red
Colchicine	Dark Brown
Cryptopine	Dark Brown
Daturine	Deep reddish violet
Ergotine	Yellowish brown
Homatropine	Yellowish brown
Hydrastine	Yellow
Hyoscyamine	Deep reddish violet
Phenacaine	Reddish brown with violet
Physostigmine	Violet to brown
Quinidine	Brown to violet on heating
Quinine	Orange-red
Scopolamine	Deep violet
Strychnine	Yellow
Veratrine	Light rose-violet

No Color Reaction.—Aconitine, brucine, caffeine, cinchonidine, cocaine, cotarnine hydrochloride, delphinine, dionine, emetine, beta-eucaine hydrochloride, gelseminine, heroine, morphine, narceine, nicotine, papaverine, pelletierine, pilocarpine, piperine, pseudopelletierine, sanguinarine nitrate, sparteine, solanine, theobromine, theophylline.

AMINO ACIDS AND DERIVATIVES

para-Aminophenylglycine	Brown
Diiodotyrosine	Yellowish brown
Glycyltryptophane	Reddish brown
Hippuric acid	Brownish yellow
para-Nitrophenylglycine	Red
alpha-Phenylalanine	Red to brown
Phenylglycine	Reddish brown, trace violet
Tryptophane	Reddish brown
Tyrosine	Yellowish brown

No Color Reaction.—Acetylphenylglycine, alpha-alanine, arginine, asparagine, aspartic acid, dl-benzoylalanine, betaine hydrochloride, creatine, creatinine, edestine, ethylglycollate, glutamic acid, glycine, isoleucine, leucine, beta-phenylalanine, dl-valine.

ALIPHATIC ACIDS

dl-alpha-Aminoacetylacetic acid	Slate
Mucic acid	Brown

No Color Reaction.—Aconitic acid, adipic acid, alpha-bromopropionic acid, beta-bromopropionic acid, formic acid, fumaric acid, levulinic acid, maleic acid, malic acid, malonic acid, mesaconic acid, palmitic acid, propionic acid, stearic acid, succinic acid, tartaric acid, tartronic acid, trichloracetic acid.

ALIPHATIC ACID SALTS, ESTERS, AND DERIVATIVES

No Color Reaction.—Ethyl oxalate, ethyl succinate, isoamyl propionate, isobutyl acetate, isobutyl isothiocyanate, methyl isothiocyanate, sodium formate, sodium oxalate, thallous formate, thallous malonate, triacetin, tributyrin.

ALIPHATIC ALCOHOLS AND KETONES

Methyl heptenone	Brown
Phorone	Brown

No Color Reaction.—Acetylacetone, cetyl alcohol, dulcitol, erythritol, ethylene glycol, isobutyl alcohol, isopropyl alcohol, mannitol, melissyl alcohol, octyl alcohol, trichlorobutyl alcohol.

SUGARS

No Color Reaction.—Arabinose, galactose, glucose, lactose, levulose, maltose, d-mannose, melezitose, raffinose, rhamnose, sucrose, xylose.

UREA AND URIC ACID DERIVATIVES

Allylphenylthiocarbamide	Reddish orange
Allylthiocarbamide	Red
Diphenylthiourea	Red
Guanine hydrochloride	Reddish orange
Peralga (Aminopyrinediethylbarbiturate)	Brownish orange
Triphenylguanidine	Red

No Color Reaction.—Allylthiourea, amytal (isoamylethylbarbituric acid), acetylmethylurea, allantoine, alloxantine, barbital (diethylmalonylurea), barbituric acid, biuret, dl-n-butylthiourea, dibromobarbituric acid, ipral (calcium ethylisopropylbarbiturate), luminol (phenylethylbarbituric acid), thiobarbituric acid, thiourea, urea, urethane, uric acid.

GLUCOSIDES

Amygdalin	Reddish brown
Phlorhizin	Dirty green
Pierotoxin	Violet
Santonin	Reddish brown, trace violet

No Color Reaction.—Aesculin, arbutin, colocynthin, convallamarin, digitalin, elaterin, salicin, saponin.

MISCELLANEOUS ALIPHATIC COMPOUNDS

Acetamide	Red
Oenanthol	Yellowish brown

No Color Reaction.—Acetal, acetaldoxime, acetoxime, aldehydeammonia, aminoguanidine bicarbonate, bromoform, terbutyl bromide, chitin, chloral hydrate, chloral urethane, chloropicrine, dimethylglyoxime, hexachloroethane, hexamethylenetetramine, iodoform, isobutylbromide, methylglyoxal sodium bisulphite, monochlorohydrine, nitrosodiethylamine, oxamide, pinacol hydrate, propionamide, sulphonal, thialdine, tribromohydrine, trichloroacetylchloride, trimethylenebromide, trional, veronal.

BENZENE AND TOLUENE DERIVATIVES

Azoxybenzene	Violet to brown to black
Mesitylene	Brown

No Color Reaction.—ortho-Bromochlorobenzene, para-bromochlorobenzene, ortho-bromonitrobenzene, meta-chloronitrobenzene, ortho-chloronitrobenzene, para-chloronitrobenzene, para-chlorotoluene, ortho-dichlorobenzene, 2-5-dichloronitrobenzene, iodosobenzene, isopropylbenzene, meta-nitrotoluene, ortho-nitrotoluene, para-nitrotoluene, styrene, para-xylenesulphonic acid.

ANILINE AND DERIVATIVES

Acetoanilide	Yellow to brown
Acetyl-ortho-methyltoluidine	Reddish brown
Acetylphenetidine	Brown
Acetyl-ortho-toluidine	Reddish brown
Acetyl-para-toluidine	Orange-brown
meta-Acetxylidine	Brown
Aminoazobenzene	Brown
2-Aminotoluene-4-sulphonic acid	Brownish black
meta-Anisidine	Reddish brown
ortho-Benztoluide	Reddish brown
para-Bromoacetanilide	Red to brown
meta-Bromoaniline	Reddish brown
para-Bromoaniline	Reddish brown
meta-Chloroaniline	Reddish brown
1, 2, 4-Dinitroaniline	Reddish brown
meta-Nitroaniline	Reddish brown with violet
para-Nitroaniline	Brown with violet
para-Nitrodimethylaniline	Bright red
Nitrosodimethylaniline	Orange-red
1, 2, 3,-Nitrotoluidine	Brown to black
1, 2, 4-Nitrotoluidine	Brown
1, 3, 4-Nitrotoluidine	Brown to black
ortho-Phenetidine	Reddish brown
para-Phenetidine	Brown
meta-Toluidine	Brown to black
ortho-Toluidine	Reddish brown
para-Toluidine	Brown
Trinitroaniline	Reddish brown

No Color Reaction.—Acetyl-para-anisidine, acetyl-para-methyltoluidine, 2-aminotoluene-5-sulphonic acid, 4-aminotoluene-2-sulphonic acid, aniline, ortho-anisidine, para-anisidine, benzanilide, para-benztoluide, ortho-bromoaniline, chloramine, ortho-chloroaniline, para-chloroaniline, 1, 2, 4-dichloroaniline, 2, 5-dichloroaniline, exalgine, meta-nitrodimethylaniline, phenyl-beta-diphenylamine, tolidine, tribromoaniline, 1, 2, 4-xylidine, 1, 3, 4-xylidine.

PHENOLS AND DERIVATIVES

Acetyl-meta-aminophenol	Reddish brown
Acetyl-para-aminophenol	Greenish brown
meta-Aminophenol	Dark brown
ortho-Aminophenol	Brown to black
para-Aminophenol	Brown to black
para-Benzalaminophenol	Dirty brown
ortho-Chloromercuriphenol	Greenish brown
ortho-Nitrophenol	Brown
Orcinol	Greenish brown
Phloroglucinol	Green
Pyrogallic acid	Brownish green
Tetrabromo-ortho-phenol	Reddish brown

No Color Reaction.—5-Benzalamino-2-cresol, benzoylthymol, para-bromophenol, carvacrol, catechol, ortho-chlorophenol, para-chlorophenol, meta-cresol, ortho-cresol, para-cresol, 3, 5-dibromo-ortho-cresol, 2, 4-dichlorophenol, dimethylhydroresorcinol, 2, 3-dinitrophenol, 2, 4-dinitrophenol, 2, 6-dinitrophenol, meta-nitrophenol, para-nitrophenol, phenol, picric acid, thymol, tribromophenol, trichlorophenol, xylenol.

AROMATIC ACIDS

Acetylsalicylic acid	Orange
dl-Aminophenylacetic acid	Dark brown
Anisic acid	Violet to rose-red
Arsanilic acid	Green
Diiodosalicylic acid	Orange
Diphenylacetic acid	Slight violet to brown
5-Iodosalicylic acid	Orange
Metanilic acid	Dark brown
Naphthionic acid	Brown

No Color Reaction.—meta-Aminobenzoic acid, para-aminobenzoic acid, anthranilic acid, benzilic acid, meta-bromobenzoic acid, ortho-bromobenzoic acid, para-bromobenzoic acid, meta-chlorobenzoic acid, ortho-chlorobenzoic acid, para-chlorobenzoic acid, cinchophen, cinnamic acid, cumaric acid, gallic acid, mandelic acid, para-mercurichlorobenzoic acid, meta-nitrobenzoic acid, ortho-nitrobenzoic acid, para-nitrobenzoic acid, quinic acid, salicylic acid, tannic acid, terephthalic acid, ortho-toluic acid, para-toluic acid.

AROMATIC ACID DERIVATIVES

Benzamide	Brown
Methyl cinnamic ester	Gray-brown
Methyl salicylate	Yellowish brown
para-Nitrobenzoyl chloride	Pink

No Color Reaction.—Benzoic anhydride, benzyl benzoate, butyl benzoate, cumarin, ethyl benzoate, ethyl salicylate, isoamyl benzoate, isoamyl salicylate, isobutyl benzoate, methyl benzoate, neocinchophen (ethyl-6-methyl-2-phenylquinoline-4-carboxylate), nicotinic acid nitrate, meta-nitrobenzoyl chloride, phenyl salicylate, phthalimide.

AROMATIC ALDEHYDES, ETHERS, ALCOHOLS, AND KETONES

para-Aminoacetophenone	Rose-violet to brown
Anisaldehyde	Rose-violet to brownish red
Benzalacetophenone	Greenish brown
Benzhydrol	Dark brown
para-Nitroanisole	Red-orange
5-Nitrosalicylaldehyde	Orange
Salicylaldehyde methyl ether	Red
Saligenin	Reddish brown
Tetramethyldiaminobenzophenone	Red
para-Tolylaldehyde	Greenish brown
Vanillin	Reddish brown

No Color Reaction.—Anisole, benzalacetone, benzophenone, ortho-bromoanisole, para-bromoanisole, ortho-bromonitrobenzaldehyde, 1, 2, 5-bromosalicylaldehyde, ortho-chlorobenzaldehyde, isophthalaldehyde, meta-methoxysalicylaldehyde, methylacetophenone, ortho-nitrobenzaldehyde, phenetole, phthalic acid aldehyde, piperonal, salicylaldehyde.

HETEROCYCLIC COMPOUNDS

Acridine	Brown
Antipyrine	Brown to black
Isatin	Reddish brown
Skatole	Brown with trace lavender

No Color Reaction.—Dimethylpyrone, furoic acid, 6-nitroquinoline, oxyquinoline, piperidine, quinaldine, quinoline, succinimide.

HYDROAROMATIC COMPOUNDS

Carvene	Brown
Carvenone	Brown

No Color Reaction.—d-Borneal, l-borneol, dl-camphor (natural), camphor (synthetic), camphoric acid, camphorsulphonic acid, limonene, menthol, quercite, terpineol, terpenyl acetate.

NAPHTHALENE AND ANTHRACENE DERIVATIVES

Acenaphthene	Brown
Acet-alpha-naphthalide	Dark brown
Acet-beta-naphthalide	Reddish brown
Alizarin	Brown
1, 5-Dinitronaphthalene	Reddish brown
Dibromoanthracene	Reddish brown
Naphthalic anhydride	Reddish brown
beta-Naphthol	Green
alpha-Naphthylamine	Slate
beta-Naphthylamine	Reddish brown
alpha-Naphthylaminoazobenzene	Dark brown
alpha-Naphthylisocyanate	Red
Nitroso-beta-naphthol	Green

No Color Reaction.—alpha-Bromonaphthalene, beta-naphthalenesulphonic acid, naphthol-methyl-alpha-ether.

MISCELLANEOUS AROMATIC COMPOUNDS

Amarine	Orange-red with violet
Benzcatechin	Grey-brown
Benzoin	Dark brown
Benzylphenylhydrazine	Brown
2, 4-Dinitrophenylhydrazine	Dirty violet with brown
Fluorene	Green to black
Isoamylphenylhydrazine	Reddish brown, trace violet
para-Nitrophenylhydrazine	Violet to blue black
Phenanthrene	Greenish brown
Phenanthrenequinone dioxime	Green to dark brown
Phenylhydrazine hydrochloride	Reddish brown trace violet
Thymolphthalein	Brown to greenish brown
para-Tolylisothiocyanate	Brown, streaks of violet
para-Tolylquinolinesulphate	Grey
Triphenylmethane	Violet to blue
Turmeric	Brown

No Color Reaction.—Abietic acid, adrenaline, benzil, diphenyl, isoeugenole, ortho-nitroacet-meta-xylidide, meta-nitrobenzhydrazide, phenolphthalein, meta-phenylenediamine hydrochloride, quercite, rheumatine (saloquinine salicylate), salvarsan, tetrabromophenolphthalein, thiosemicarbazide, ortho-tolunitrile, para-tolunitrile, para-tolylthioquinanthrene.

The color reactions for the tests where an equal amount of impurity was added to the atropine were recorded, and also the reactions where five times the amount of impurity was added; but the listing of these would require too much space. Therefore, only those compounds which caused the violet color to be completely masked will be given. Of the remainder of the compounds, many gave no interference, whereas others gave more or less interference.

Organic compounds which completely cover up the Vitali test for atropine when present in equal amounts were:

Acet-alpha-naphthalide, acet-beta-naphthalide, acetyl-para-anisidine, acetyl-ortho-methyltoluidine, acetylphenetidine, alizarine, allylphenylthiocarbamide, allylthiocarbamide, para-aminoazobenzene, meta-aminobenzoic acid, para-aminobenzoic acid, para-aminophenol, 2-aminotoluene-4-sulphonic acid, 2-aminotoluene-5-sulphonic acid, benzanilide, para-bromoacetanilide, cumaric acid, edestin, glycyltryptophane, hippuric acid, 5-iodosalicylic acid, isoeuginol, beta-naphthol, alpha-naphthylisocyanate, para-nitro-dimethylaniline, meta-nitrophenol, ortho-nitrophenol, peralga, phenanthrene, phenanthrene quinone dioxime, para-phenetidine, phenolphthalein, salvarsan, tetrabromophenolphthalein, tetramethyldiaminobenzophenone, trinitroaniline, triphenylguanidine, tryptophane, vanillin.

Organic compounds, in addition to those listed above, which completely cover up the Vitali test for atropine when present in amounts five times that of atropine were:

Abietic acid, meta-acetxylidine, acetyl-meta-aminophenol, acetyl-para-aminophenol, acetyl-ortho-toluidine, acetyl-para-toluidine, aesculine, amarine, para-aminophenylglycine, 4-amino-toluene-2-sulphonic acid, para-anisidine, anthranilic acid, apomorphine, arsanilic acid, benzalamino-2-cresol, para-benzalaminophenol, benzoin, benztoluid, alpha-bromonaphthalene, brucine, ortho-chloromercuriphenol, codeine, meta-cresol, cryptopine, diiodosalicylic acid, 1, 5-dinitronaphthalene, 2, 4-dinitrophenol, diphenylthiourea, emetine, fluorene, guanine hydrochloride, hydrastine, isatin, isoamylphenylhydrazine, isoamylsalicylate, metanilic acid, morphine, naphthionic acid, alpha-naphthylamine, alpha-naphthylaminoazobenzene, narceine, para-nitrophenol, para-nitrophenylglycine, nitroso-beta-naphthol, 1, 2, 3-nitrotoluidine, 1, 2, 4-nitrotoluidine, orcinol, ortho-phenetidine, phenylglycine, phloroglucinol, phloridzin, physostigmine, piperine, rheumatine, salicin, salicylaldehydemethylether, santonin, skatole, tetrabromo-ortho-cresol, thymol, thymolphthalein, ortho-toluidine, ortho-tolunitrile, para-tolunitrile, tribromoaniline, tyrosine, 1, 3, 4-xylidine.

From a study of the preceding data, it will be noted that there were twenty-seven organic substances which gave various shades of violet, lavender, or purple, which might, to some degree, be confused with the Vitali test for atropine. Van Urk[3] reported the following to give some shade of violet, but our work did not show any violet color in the test: phenylglycine, ortho-aminophenol, pyrogallic acid, and aesculine.

In general, the compounds giving a similar atropine test do not belong to any definite group of organic compounds, nor does any special organic radical seem to be responsible for the characteristic test. Nearly all of them, however, contained nitrogen in some form.

There were a number of organic compounds which partially covered up the atropine test. Of course, in a carefully conducted analysis, many of these substances would be removed by means of the different organic solvents.

CONCLUSIONS

1. A large number of organic compounds have been subjected to Vitali's test. Some of these have been found to give a color reaction similar to atropine.

2. The interferences of organic compounds when present in varying amounts with Vitali's test for atropine have been determined.

REFERENCES

1. Vitali, D.: J. d. méd. chir. et pharm. Bruxelles. **71**: 66, 161, 167, 279, 382. See Witthaus, Manual of Toxicology, 1911.
2. Witthaus, R. A.: Manual of Toxicology, 1911.
3. Van Urk, H. W.: Behavior of Various Aromatic Substances in Vitali's Reaction, Pharm. Weekblad **62**: 926, 1925. See Chem. Abs. **19**: 3561, 1925.
4. Celsi, S. A.: Studies on the Vitali Reaction for the Detection of Atropine, Ann. Farm. Bioquim. **1**: 140, 1930; **2**: 26, 1931. See Chem. Abs. **25**: 1636; **26**: 1710.
5. Fuller, H. C.: Notes on Two Important Alkaloidal Reactions, Bureau of Chem. Bull. **150**: 36, 1912.
6. Poe, C. F., and O'Day, D. W.: A Study of Mandelin's Test for Strychnine, J. Am. Ph. Assn. **19**: 1292, 1930.

7. Poe, C. F., and Stehley, Pearl S.: A Study of Fröhde's Test for Morphine, J. LAB. & CLIN. MED. **18**: 375, 1933.
8. Levine, V. E.: Studies in Toxicologic Chemistry, J. LAB. & CLIN. MED. **11**: 809, 1926.
9. Levine, V. E., and Magiera, E. A.: Studies in Toxicologic Chemistry, J. LAB. & CLIN. MED. **12**: 773, 1927.

A MODIFIED WITT FILTRATION APPARATUS SUITABLE FOR MULTIPLE POTASSIUM DETERMINATIONS*

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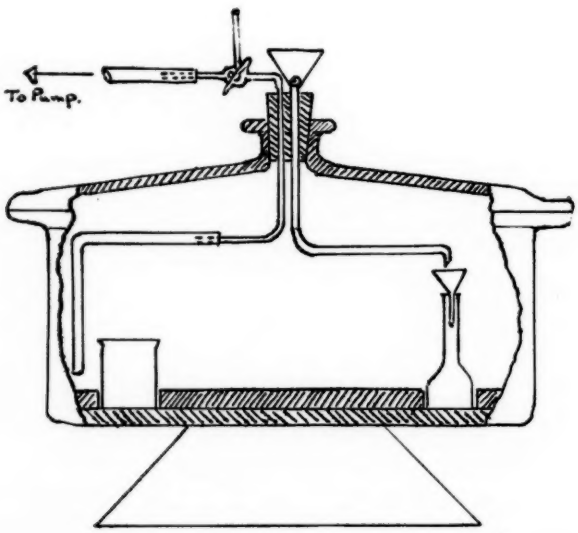
A SIMPLE modification of the micro-filtration apparatus described by Shohl[1] has been found very useful in the determination of the potassium content of biologic fluids. By means of this modified apparatus multiple determinations of potassium may be made without the necessity for the removal of the upper portion of the chamber thus avoiding repeated reexhaustion of the air from the desiccator.



The apparatus, as modified, consists of a large desiccating jar provided with a tightly fitting rubber stopper bored with two holes. Through one passes the tube connected with the water pump. The distal end of this tube is extended by a short piece of pressure tubing so that upon readmission of air to the apparatus it enters near the base of the chamber. Through the other hole in the rubber stopper is inserted the small glass funnel containing the asbestos mat as described by the above author. The stem of this funnel is bent at right angles and the tip again bent at right angles so as to allow clean drainage. A thick wooden block provided with a series of six holes, each large enough to accommodate the base of a 25 c.c. volumetric flask and bored so that the holes lie along the circumference of a circle equal in

*From the Laboratory of Pharmacology, University of Michigan Medical School.
Received for publication, July 9, 1932.

radius to the length of the horizontal portion of the funnel-stem, is placed on the floor of the desiccator. Two larger holes, also on this circumference, are provided to accommodate beakers for the reception of the excess chloroplatinic acid and the solutions used for washing the filtrate respectively. By rotation of the funnel its stem may be brought over any of the volumetric flasks or either of the beakers as desired. The time thus saved is considerable.

REFERENCE

1. Shohl, Alfred T.: Apparatus for Micro Filtration, J. Am. Chem. Soc. 1: 417, 1928.

CONCERNING THE STABILITY OF GLYCERINATED HEMOLYSIN*

ROBERT A. KILDUFFE, M.D., ATLANTIC CITY, N. J.

IN A previous communication† I reported that antisheep hemolysin to which an equal volume of chemically pure glycerin had been added showed practically no loss in titer after a period of seven years.

The hemolysin in question prepared in 1916, had an original titer of 1:20,000 when titrated in accordance with the method described for use in Kolmer's quantitative complement-fixation test.

During my service with the A. E. F. from 1917 to 1919 the hemolysin was kept in the refrigerator. It was later shipped by freight from Philadelphia to Pittsburgh, thus being exposed to summer temperature for some time; still later again shipped by freight to Los Angeles, also during summer temperature; refrigerated eighteen months and again shipped by freight from Los Angeles to Philadelphia, and finally, to Atlantic City where it was again refrigerated.

At varying intervals the hemolysin has been titrated according to Kolmer's method, with the results tabulated below:

Original titer, 1916	:	1:20,000
Titer, 1920	:	1:18,000
Titer, 1923	:	1:16,000
Titer, 1932	:	1:20,000

In each instance the titer was read as the smallest quantity of hemolysin giving complete, sparkling hemolysis with 0.5 c.c. of pooled 1:30 complement and 0.5 c.c. of 2 per cent cell suspension.

The last titration was conducted with sheep cells of normal fragility test reaction.

The variations, in view of the last result, were apparently related to variations in complement activity rather than to variations in the hemolysin.

It appears, therefore, that glycerinated hemolysin may be preserved for a period of sixteen years without special precautions as to consistent temperature.

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*From the Laboratories of the Atlantic City Hospital.
Received for publication, June 23, 1932.

†Kilduffe, R. A.: A Note Upon the Stability of Preserved (Glycerinated) Antisheep Hemolysin, J. LAB. & CLIN. MED. 9: 652, 1924.

GIEMSA STAIN FOR TISSUE, RAPID METHOD*†

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THE greatest drawback to the use of the Giemsa stain in the preparation of histologic sections is the length of time required. The usual procedure consumes from eight to twelve hours with two or more changes of the staining solution. The following modification is in use at this hospital and the results are found to be as satisfactory as those obtained with the longer procedures.

1. Fix tissue in Zenker's fluid forty-eight hours or more.
2. Wash in running water twenty-four hours.
3. Dehydrate, clear and embed in paraffin.
4. Cut sections 4 to 6 microns in thickness.
5. Treat sections with xylol followed by graded alcohols.
6. Treat with Lugol's solution (5 c.c. to 100 c.c. distilled water) for thirty minutes followed by one change of 95 per cent alcohol and one of tap water.
7. Treat sections for ten minutes in a 0.5 per cent solution of sodium hyposulphite.
8. Wash well in tap water.
9. Stain for fifteen minutes in the following solution:

Giemsa stain (Stock solution)	10 c.c.
Acetone C. P.	10 c.c.
Methyl Alcohol C. P.	10 c.c.
Sol. Sodium Carbonate 0.5 per cent	2 M
Distilled Water	100 c.c.

10. Wash rapidly in water and differentiate, using the method employed by Dr. S. B. Wolbach, as follows:

Treat sections individually in two changes of the following solution:

Colophonuim	15 gm.
Acetone	100 c.c.

Renew the solution as soon as the precipitated colophonium fails to dissolve quickly. Differentiation usually takes place in from fifteen to forty-five seconds.

11. Pass sections through a solution of:

Acetone	70 c.c.
Xylol	30 c.c.

Follow with xylol and mount in oil of cedarwood.

*From the Clinical Laboratory and Laboratory Center and Cancer Center, Edward Hines, Jr., Hospital.
Received for publication, July 2, 1932.
†Published under R & P 6969 U. S. Veterans' Administration.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CHOLESTEROL, Blood, In Thyroid Disease. Arch. Int. Med. **51**: 22, 1933.

The blood cholesterol during fasting was determined as a routine in 505 cases of thyroid disease, along with the pulse, the weight, and the basal metabolic rate.

The lowest average values for blood cholesterol are found in patients in or near thyroid crises.

Auricular fibrillation in toxic goiter is associated with the next lowest average level of cholesterol.

The average cholesterol value in all types of exophthalmic goiter is lower than in toxic adenomatous goiter.

Recurrent hyperthyroidism is associated with cholesterol values that are almost as low as those in exophthalmic goiter.

The average cholesterol value in nontoxic goiter is normal, although the scatter is wider than expected. Age appears to raise the blood cholesterol in these cases.

Chronic thyroiditis is associated with higher average values than in any other thyroid disease except myxedema.

The level of the blood cholesterol and the basal metabolic rate bear a reciprocal relationship when judged by average values.

SEMINAL STAINS, Modification of Barberio's Test, Harrison, G. A. Lancet **2**: 940, 1932.

A portion (1 by 1 cm.) of material containing the stain is pushed into a conical centrifuge tube, 1 c.c. of 2.5 per cent solution of trichloracetic acid added and the tube well shaken at intervals. After standing for one hour, the tube is centrifuged and the supernatant fluid discarded. An equal volume of saturated solution of trinitrophenol is then added when, in the presence of spermine, a precipitate forms. The tube is then placed in a boiling water bath until solution occurs and allowed to cool slowly in the bath. When cool, centrifuge if necessary and examine the crystals microscopically. The presence of crystals indicates a positive reaction, amorphous particles are without significance. A *copious* amorphous deposit may be due to a high concentration of spermine picrate. In this case dilute with one or more volumes of an equal mixture of 2.5 per cent solution of trichloracetic acid and saturated solution of trinitrophenol and heat and cool as before.

CARBON MONOXIDE: "Normal" Content of Blood, Gettler, A. O., and Mattice, M. R. J. A. M. A. **100**: 92, 1933.

The average content of carbon monoxide in the blood of eighteen persons living in New York City under conditions of minimal exposure was found to be 0.27 volumes per cent. This represents about 1.0 to 1.5 per cent of the hemoglobin combined with carbon monoxide.

The average content of carbon monoxide in the blood of twelve persons confined to a state institution in an ideal rural locality was found to be 0.24 volumes per cent. Most of these showed a hemoglobin saturation of less than 1 per cent.

The average content of carbon monoxide in the blood of twelve New York City street cleaners was found to be 0.69 volumes per cent. This represents about 3 per cent saturation of the hemoglobin with carbon monoxide.

Two taxicab drivers were found to have on several occasions a carbon monoxide content ranging from 1.47 to 4.33 volumes per cent. This represents a hemoglobin saturation of 8.0 to 19.0 per cent.

Tobacco smoking appreciably increases the carbon monoxide in the blood and cannot be ignored in the interpretation of laboratory results.

B. TUBERCULOSIS: Concentration Method For, Frigimelica, C. Policlinico 39: 1776, 1932.

The sputum is first shaken with a few centimeters of water in a beaker and then sufficient saturated aqueous solution of trinitrophenol added to saturate the specimen.

The mixture is then stirred with a glass rod until it is thoroughly stained a bright yellow.

Then, with gentle heat, add 10 per cent sodium hydroxide until the sputum dissolves. Cool and add one-third volume of chloroform, shake vigorously and centrifuge ten minutes. The bacilli are concentrated in the whitish ring formed at the zone of separation of the chloroform.

HEMOGLOBIN, Standards, Haden, R. L. Am. J. Clin. Path. 3: 85, 1933.

From a thorough survey the author concludes that:

In making hemoglobin determinations only an instrument or method should be employed in which the results are recorded in grams as determined by the oxygen capacity or iron content method.

For clinical purposes the reports of hemoglobin are best given in percentage of normal of a healthy adult with a red cell count of 5,000,000 cells.

The Haldane and Williamson standards can not be so transposed since in determining the standards no red cell counts were done.

There is a wide variation in hemoglobin and erythrocyte counts and consequently in the hemoglobin coefficient in the three large series (Haden, Osgood and Haskins, and Wintrobe) reported for normal men and women.

A satisfactory standard must give a color index within normal limits (0.90 to 1.10) in normal individuals.

In the present state of confusion, the hemoglobin coefficient should be determined for each laboratory and the hemoglobin should be reported only in percentage of this normal.

The hemoglobin percentage of a given blood when determined by this method is necessarily the same in all laboratories within the limits of technical error.

BLOOD CHEMISTRY: Comparative Value of Monochlorbenzene and Thymol When Used With Fluoride as Preservatives of Blood For Chemical Analysis, Lewis, R. C., and Mills, G. C. Am. J. Clin. Path. 3: 17, 1933.

The following is a summary of the results of this study:

A combination of monochlorbenzene and potassium fluoride was found to be a more effective preservative for blood sugar, total nonprotein nitrogen, and urea than thymol and potassium fluoride. Little change occurs in the uric acid and the creatinine with either preservative combination.

Blood specimens may be transported through the mails in journeys up to eight days with no appreciable change in either the blood sugar or the nitrogenous blood chemical constituents when a preservative combination composed of 0.275 gm. of potassium fluoride and 0.2 gm. of monochlorbenzene per 20 c.c. of blood is employed. In journeys of twelve days, the sugar is well preserved, and only slight changes occur in the nonprotein nitrogenous constituents.

Neither monochlorbenzene-potassium fluoride nor thymol-potassium fluoride will satisfactorily preserve blood specimens which are subjected to a temperature of 37°C. for twenty-four hours or longer.

If a blood specimen treated with monochlorbenzene-potassium fluoride has been subjected to high temperatures during passage in the mails, there will be a failure of preservation of the blood chemical constituents. Such blood will contain numerous small clots which will interfere with pipetting of the blood. The absence of such clots, therefore, offers a simple criterion as to successful preservation.

When preserved by the addition of 0.2 gm. of monochlorbenzene to 20 c.c. of blood,

oxalated blood specimens may be stored in a refrigerator at 6°C. for ninety-six hours with practically no variation in the blood chemical constituents.

ANTIGENS, Flask for Dilution of, Hinton, W. A. Am. J. Clin. Path. 3: 41, 1933.

In view of the necessity for accurate dilution of antigens and the impossibility of eliminating the personal equation in the preparation of antigen suspensions, the author suggests that the dilutions be made in specially prepared flasks.

An inverted V-shaped partition is blown in the bottom of an ordinary Erlenmeyer flask producing two semicircular compartments. In 25 to 150 c.c. flasks the compartments hold from 3 to 5 c.c. and from 8 to 10 c.c. in the larger sizes (125 to 500 c.c.).

The antigen is pipetted into one compartment and the diluent into the other; mixture is made by shaking the flask quickly from side to side.

TUBERCULIN TESTS, Results With Different, Friedman, E., Black, M. H., and Esserman, A. L. Am. J. Dis. Child. 45: 58, 1933.

A study of the various diagnostic tuberculin cutaneous tests, performed with scrupulous regard for details, demonstrated the utter unreliability of the Pirquet and the modified Moro tests.

It failed to substantiate the claims advanced by other observers for the "multiple puncture" method.

This study demonstrated the superiority of the Mantoux and "single puncture" technic.

The recommendation appears warranted that these two tests supersede the others.

For routine office and bedside work the "single puncture method" may be given precedence, because it is easier to perform, is less time-consuming, eliminates the necessity for dilution and refrigeration, is less expensive and, withal, yields results as accurate as those obtained by the well established Mantoux test.

MENINGITIS, Tuberculous, Laboratory Diagnosis of, Foard, A. G., and Forsyth, A. Am. J. Clin. Path. 3: 45, 1933.

This study is thus summarized:

In the diagnosis of tuberculous meningitis from cerebrospinal fluid, the findings of tubercle bacilli on smear or culture of the production of tuberculosis in a guinea pig injected with the fluid is conclusive evidence of the disease. However, by the last two means the diagnosis can rarely, if ever, be made before the patient is dead. Smear examination if done on repeated samples should reveal bacilli in 80 to 100 per cent, depending on the technic and persistence of the examiner. But in the absence of a positive smear the following findings, especially when they are all present (and they usually are), should warrant the tentative diagnosis of tuberculous meningitis; (1) increased cell count, averaging about 200, producing a slight ground-glass appearance in the fluid; (2) Differential count usually shows marked preponderance of lymphocytes but may show a moderate number of polys in smear-negative cases; (3) Strong tests for globulin (2 to 4); (4) the formation of an inverted pine tree web on standing for several hours or overnight; (5) colloidal gold curve showing maximum precipitation in the sixth or seventh tube; (6) the sugar content markedly or moderately reduced; averaging about 36 mg. per 100 c.c. fluid, and (7) a decrease in chlorides below 650 mg. Especially should the association of a marked decrease in sugar accompanied with a noteworthy decrease in chlorides in fluids not showing characteristics of a suppurative meningitis be stressed as a strong presumptive sign of tuberculous meningitis.

INTESTINAL INFECTIONS, In Infants, Johnston, M. A., Brown, A., Tisdall, F. F., and Fraser, D. T. Am. J. Dis. Child. 45: 1, 1933.

To test the validity of the various hypotheses as to the cause of acute intestinal intoxication, 172 patients with acute intestinal intoxication or related diagnoses and 107 controls were subjected to clinical investigation as well as bacteriologic survey of the flora of the upper respiratory tract and gastro-intestinal tract. Although evidence of late involvement of the middle ear developed in 63 per cent of the cases, there was similar involvement

in over 60 per cent of the controls who showed no evidence of acute intestinal intoxication. Hemolytic streptococci were isolated very rarely from the ear, nose, and throat in patients either during life or post mortem. Hemolytic streptococci were isolated more frequently from controls both during life and post mortem, although these showed no symptoms suggestive of acute intestinal intoxication.

Evidence was found bacteriologically or serologically of infection with pathogenic micro-organisms of the colon-paratyphoid-dysentery group.

It was concluded that acute intestinal intoxication is not caused by masked mastoid infection.

Acute intestinal intoxication, fermentative diarrhea, infectious diarrhea, dysentery and summer diarrhea are synonyms indicating one condition, a gastro-intestinal infection with species of the colon-paratyphoid-dysentery group of bacilli.

LIVER FUNCTION, Rose Bengal Test of, Stowe, W. P., Delprat, G. D., and Weeks, A. Am. J. Clin. Path. 3: 55, 1933.

The following results are reported:

Normal values (17 cases) 85 to 110 per cent.

Gall bladder disease: rose bengal elimination was in inverse relationship to the acuteness of the disease.

In three normal pregnancies the function varied from 85 to 100 per cent; early preeclamptic toxemia (1) 80 per cent; severe eclampsia (1) 55 per cent; hyperemesis gravidarum (2) 55 and 40 per cent.

Cirrhoses gave uniformly low values.

Chronic alcoholism (4) 100, 80, 90, and 42 per cent.

Carcinoma, secondary (3) 78, 80, and 40 per cent.

Diffuse hepatic damage (3) 60, 67, and 57 per cent.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Some Factors in the Localization of Disease in the Body*

AMONG the many and varied phenomena of disease, those concerned with the localization of pathologic processes within the body present a problem, as yet unsolved, of great as well as practical interest.

As stated in the introductory chapter of this book: "When aggressive agents have entered the body the immediate safety of the individual usually is ensured if the aggressors can be retained or collected into a circumscribed region where the integrity of the tissues is not a vital necessity, and where the powers of resistance may be exercisd in concentrated force."

The purpose of this book is "to elucidate the circumstances in which such segregation and retention come about, and to determine by what artifical means these defensive processes may be assisted."

It is obvious that this is, indeed, a complex problem not to be easily solved and that to its ultimate elucidation must be bent the work of many investigators in varied fields.

The author has made a distinct and important contribution based upon extensive experimental studies and a careful review and discussion of the literature germane to the subject.

As a basis for his discussion he has studied in detail the localization of colloids, larger organic particles, viruses, and micro-organisms from the blood stream and upon these studies has founded the general principles he advances.

For the commonly used phrase, "increased permeability of the blood vessels" he substitutes a new word, "diapiresis," coined by D'Arey Power and signifying the passage of colloidal or other particles of suspended matter, including bacteria or blood cells, through the unbroken walls of the blood vessels.

His studies of inflammatory processes may be thus summarized:

1. Inflammation is the common, though not the only, cause of increased endothelial permeability.

2. The essential feature of inflammation is an increased permeability of the injured cell.

3. The secondary manifestations of inflammation as seen in the higher animals vary widely with the duration, degree, and nature of the irritant.

4. A cell, though surviving an injury, does not always recover completely, it may remain in a condition of abnormal permeability.

5. Diapedesis is a passive process, being independent of activity on the part of the leucocytes.

6. Not only blood cells, but inert particles also, are transported through the capillary endothelium under the influence of inflammation.

The transudation of normal proteins is rendered possible by an increased permeability of the vascular endothelium, to which a number of secondary factors contribute influencing the rate of transudation. Among these are: hyperemia, an increased hydrostatic intracapillary pressure, osmotic pressure, diffusion currents, and electrophoresis.

The factors concerned in the production of the capillary endothelium are thus summarized: inflammation, anoxemia, and accumulation of metabolites, increased P_H of the blood or tissues, poisons present in the circulating blood, possibly quantitative defects in the constitution of the

*Some Factors in the Localization of Disease in the Body. By Harold Burrows, Later Hunterian Professor, Royal College of Surgeons. Cloth, 299 pages, 6 figures, 8 colored plates. Wm. Wood and Co., New York.

blood, certain cellular degenerations, increased physiologic activity, perhaps an immaturity of the vascular endothelium, and possibly an imperfect recovery of endothelium following injury.

As a result of his investigations Burrows concludes that for the localization of many blood-borne diseases three conditions are required: an abnormal permeability of the small blood vessels, the presence of forces which will transport the noxious agents through the endothelial cytoplasm, and the retention of noxious agents in the tissues under the influence of inflammation, all of which lead to the localization, not only of the agents of disease, but to the factors of defense.

This volume represents a noteworthy contribution to the study of disease and well repays perusal and study.

Endocrine Medicine*

BECAUSE of an ever increasing knowledge of the functions of the various endocrine glands, it may be conservatively said that this speciality is now looked upon as an integral and indispensible part of modern medicine. Interest in this subject has been widespread as well as intensive, and as a result fundamental discoveries have followed one another in rapid succession during the past fifteen years. Naturally there has been considerable interspersed conjecture and theorizing, and much has been written that was later disproved or discarded.

Engelbach's "Endocrine Medicine" is an up to date, conservative but comprehensive appraisal of the known facts concerning the endocrine glands. This work comprises three volumes. The author has approached his treatise in a logical, sequential and natural manner, replacing dogmatism with known facts, and thereby treating the reader to a freedom of thought that is both healthy and refreshing.

The first volume concerns itself for the most part with fundamentals. There is a fairly comprehensive account of the history of endocrinology in the opening chapters. Organology, physiology, nomenclature and etiology are adequately covered, and diagnostic procedures are thoroughly discussed. The relation of the endocrinopathies to general medicine, the specialties and to public health is frankly considered.

The second volume is taken up with a thorough consideration of the infantile and juvenile endocrinopathies.

Adolescent and adult endocrine disturbances are treated, in the third volume, with a painstaking and meticulous care for detail characteristic of the entire work. This volume alone contains 862 pages.

There is a foreword by Dr. Lewellys F. Barker. The entire work is profusely illustrated, and there are many helpful and interesting charts as well.

It is quite evident that the author was a master of his subject but in no instance has zealousness been substituted for fact.

This should be an ideal source of information or a reference work for either the student, the specialist, or the internist.

Skin Diseases and Nutrition†

THE German edition of this book was reviewed in the November, 1932, number of the Journal. For those who cannot read German, the English translation which is now available is excellent.

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*Endocrine Medicine. By William Engelbach, M.D., F.A.C.P., B.S., M.S., D.Sc., Professor of Clinical Medicine, St. Louis University School of Medicine, 1911-24; Physician-in-Chief, St. John's Hospital, 1909-24; Member of Staff St. Louis, City, Jewish, Baptist Sanitarium, and Maternity Hospitals; President of Association for Study of Internal Secretions, 1922-23; President of the St. Louis Medical Society, 1918; Fellow of American Medical Association and American College of Physicians, Member Missouri, Illinois, New York and Southern Medical Societies. With a Foreword by Lewellys F. Barker, Professor Emeritus of Medicine, The Johns Hopkins University School of Medicine. Three Volumes and an Index Volume with 933 Illustrations. Volume I. General Considerations. Volume II. The Infantile Endocrinopathies. The Juvenile Endocrinopathies. Volume III. The Adolescent Endocrinopathies. The Adult Endocrinopathies. Springfield, Ill. Charles C. Thomas, Baltimore, Md., 1932.

†Skin and Nutrition Including the Dermatoses of Children. By Erich Urbach, M.D. Authorized English Translation by Frederick Rehm Schmidt, A.B., M.D. With 55 Illustrations, 8 Diagrams and 10 Tables. Cloth. Pages 242. Wilhelm Maudrich, Publisher, Vienna, 1932.

The Renal Lesion in Bright's Disease*

THE kidney has been one of the chief stumbling blocks for the morphologic pathologist. After nearly a hundred years from the first description of the malady by Bright there is still no unanimity concerning the character of the local lesion, or general agreement in clinical classification. The dictum "conclusions drawn from the urine are as brittle as the urinal" had its basis in careful observation and most of those interested in the pathology of Bright's Disease have long since discontinued efforts to prognosticate the character of the lesion from the study of the urine. Casts may be very numerous at one time and few or absent at another time even though in the latter instance the disease may have progressed seriously.

Addis, realizing that the reason for this variability was not in the kidneys but in variations in the concentration of the urine, such as hyalin casts dissolved in a very dilute or alkaline urine, inaugurated a quantitative study of the cellular elements of the urine.

By taking precautionary measures to provide a relatively concentrated, acid urine, he has been able to make reliable quantitative microscopic observations on twelve hour urine specimens. Space will permit only a very brief mention of his many interesting observations. He found that in twelve hour night collections made with the precautions mentioned, the normal cast excretion ranged up to 5000 per twelve hours. The normal average erythrocyte excretion was 65,750; leucocytes and epithelial cells, 322,550.

Years ago Sir James Mackenzie urged the desirability of studying individuals over years of life and correlating the clinical observations with autopsy findings. This monograph represents an achievement of this sort. Addis has followed his patients over periods up to ten years, making very careful life studies and Oliver has provided the morphologic postmortem observations. The monograph consists in an effort to correlate the two.

The authors produce very satisfactory evidence in support of their contention that conclusions drawn from the urine when the urine is properly examined may be of distinct diagnostic significance. They divide clinical Bright's Disease into three varieties, hemorrhagic, degenerative and arteriosclerotic. The pathologic findings in the kidney are classified as glomerulitis, parenchymal degeneration, interstitial proliferation and arteriosclerosis or endarteritis.

Briefly, in hemorrhagic Bright's Disease there is always a glomerulitis at autopsy. There may or may not be any or all of the other three pathologic lesions. In degenerative and arteriosclerotic Bright's Disease, there is no glomerulitis. In the degenerative form there is always parenchymal degeneration and may or may not be interstitial proliferation and sclerosis. In the arteriosclerotic form there is always interstitial proliferation and arteriosclerosis which may or may not be accompanied by parenchymal degeneration.

The authors prefer the term Bright's Disease to nephritis and nephrosis, feeling that in our present unsatisfactory classification of the pathologic lesion, the original term is less descriptively binding and therefore more desirable.

The monograph contains a wealth of interesting detail.

Handbook of Bacteriology†

THIS, the third edition of a well known manual, has undergone a thorough revision throughout and presents the subject in a thorough manner and in conformity with the latest advances in the field of modern bacteriology.

While primarily intended for the use of students this book well deserves, like its predecessors, a place in the reference library of laboratory workers as well as physicians in general.

It can be highly recommended.

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*The Renal Lesion in Bright's Disease. By Thomas Addis, Professor of Medicine, Stanford University, and Jean Oliver, Professor of Pathology, Long Island College of Medicine; formerly Professor of Pathology, Stanford University. With 170 full-page plates, 2 in color, 21 text illustrations and one folding table, cloth, pages 628. Paul B. Hoeber, Inc., New York, 1931.

†Handbook of Bacteriology. By J. W. Bigger, Professor of Bacteriology and Preventive Medicine, University of Dublin, etc. Cloth, 459 pages, 84 figures, William Wood and Co., New York.

Manual of Clinical and Laboratory Technic*

THIS small book is intended for the practical guidance of the student and interne in the systematic study of patients.

Only such technical details as properly fall within their field are given in a succinct manner. That the volume has reached a fourth edition is an evidence of its practical usefulness.

Aids to Bacteriology†

THIS is one of a series "Student Aids," small, compact volumes, presenting succinct yet comprehensive and practical surveys of specific subjects.

The present edition has been extensively and thoroughly revised bringing the book in line with the newer developments in the field of bacteriology.

While the discussions are necessarily brief the book supplies a satisfactory survey of the subject.

P_H and Its Practical Application‡

AS SAID by the authors in their introductory chapter, although the determination of hydrogen ion concentration first became the subject of investigation nearly half a century ago, it is only within the last ten or twelve years that it has become of general interest and application, a natural development arising from the evolution of relatively simple and practical methods now familiar to and utilized by laboratory and technical workers in a variety of fields.

This book is not intended to serve as a manual of technic, but rather as a handbook for the practical technologist who desires a working knowledge of the practical applications of P_H measurements.

That this purpose is achieved, and that the book, though small, is comprehensive in character is indicated by the following summary of its contents:

The first section of five chapters comprises a discussion of the mechanism of hydrogen ion determinations and, despite the inherent complexities of the subject, is clear, readable, and understandable.

The second section, of fourteen chapters, discusses and illustrates the practical application of P_H determinations in many fields such as the regulation of water supplies, water corrosion problems, disposal of sewage and industrial waste, sugar industry, gelatin and glue, leather manufacture, textile industries, cleaning processes, electroplating, general industrial chemistry, bacteriology, pathology, and titration procedures, and soils.

The volume is well printed and thoroughly indexed and can be recommended to those interested in the many and diverse problems discussed.

Handbook for Senior Nurses and Midwives§

THIS, the second edition of a manual first introduced in 1926, has been extensively revised and includes four new chapters.

The book is divided into five sections: I, Medical; II, Surgical; III, Children; IV, Obstetrical; and V, Gynecological.

The volume is addressed more to the graduate nurse and experienced midwife than to the student and, consequently, discusses the subjects covered in some detail.

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*Manual of Clinical and Laboratory Technic. By Herman B. Weiss, Associate Professor of Medicine, University of Cincinnati, and Raphael Isaacs, Associate Professor of Medicine, University of Michigan, Fourth Edition, Reset. 117 pages, with Diet Table. Cloth. W. B. Saunders Co., Philadelphia and London, 1932.

†Aids to Bacteriology. By Wm. Partridge, Public Analyst County of Dorset, etc. Cloth, 311 pages, fifth edition. William Wood and Co., New York.

‡P_H and Its Practical Application. By Frank R. LaMotte, William R. Kenny, and Allen B. Reed. Cloth, 255 pages, 18 charts, and numerous tables. Williams and Wilkins Co., Baltimore, Md.

§Handbook for Senior Nurses and Midwives. By J. K. Watson, Late House Surgeon Essex and Colchester Hospital. Cloth, 676 pages, 297 figures. Oxford University Press.

The Pathogenic Streptococci*

THIS volume VII of the Annals of the Pickett-Thomson Research Laboratories, is a continuation of the studies presented in preceding volumes of the series, of the rôle of the streptococcus group in the production of specific diseases.

In this book the rôle of the streptococci in erysipelas, skin diseases, and measles are presented in comprehensive detail embodying an extensive survey of the literature, which, in itself, is of great value.

The accompanying microphotographs as usual are excellent and excellently reproduced.

This book, like its predecessors, will be received with great interest by bacteriologists and laboratory workers and constitutes a distinct and important contribution to an, as yet, not entirely settled subject.

The Anatomy of the Human Orbit and Accessory Organs of Vision†

THE first edition of this work published in 1921 was a valuable book and, so far as this reviewer knows, the only one of its kind in the English language.

This timely second edition is enriched by exactly that amount of knowledge which the world has contributed to the subject in the past eleven years, for as the author modestly says, ". . . . over 190 papers published since 1921 have been read and incorporated." The general plan of the book is not altered, but the new material falling in its proper place makes a thorough revision, while adding but 39 pages to the volume.

Seventeen illustrations, some in colors, have been added. Another pleasing feature is the increased number of paragraph titles in bold-faced type which, in effect, serve as an index as one turns the pages. The conventional index is in the usual location.

The book deserves nothing but praise. The first edition was a good friend, much used and loaned; a necessary item in every ophthalmologist's library.

Manual of Surgery‡

THIS, the eighth edition of a well known manual represents the position of present-day surgery and in particular the teaching of the Edinburgh School.

It will undoubtedly receive the same favorable reception accorded to its predecessors.

Protoplasmic Action and Nervous Action§

IN THIS second edition, materials and bibliography are brought up to date and cognizance taken of recent developments in this field.

The book, largely based upon lectures delivered in Clark University and in the Marine Biological Laboratory, is concerned with the phenomena of reactivity and response in living matter and is essentially a treatise on the physico-chemical basis of the more general properties of living matter.

It may be regarded as a well planned and well executed exposition of the subject by a prominent worker in this field.

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*The Pathogenic Streptococci: The Rôle of the Streptococcus in Erysipelas, Skin Diseases, and Measles. Annals of The Pickett-Thomson Research Laboratory. Vol. VII, Paper. 441 pages, 35 microphotographic plates. Williams and Wilkins Co., Baltimore, Md.

†The Anatomy of the Human Orbit and Accessory Organs of Vision. By S. E. Whitman, Professor of Anatomy, McGill University, Montreal. Second edition, Cloth, 467 pages, 212 figures. Oxford University Press.

‡Manual of Surgery. By Alexander Miles, Consulting Surgeon, Royal Infirmary, Edinburgh, and D. P. D. Wilkie, Professor of Surgery, University of Edinburgh. Cloth, eighth edition, 574 pages, 176 figures. Oxford University Press.

§Protoplasmic Action and Nervous Action. By R. S. Lillie, Professor of Physiology, University of Chicago. Cloth, 417 pages, Second edition. University of Chicago Press.

The Journal of Laboratory and Clinical Medicine

VOL. XVIII ST. LOUIS, MO., APRIL, 1933 No. 7

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

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Entered at the Post Office at St. Louis, Mo., as Second-Class Matter. Additional entry as Second-Class Matter at Fulton, Mo.

EDITORIAL

The Blood Serum Proteins

THE Kjeldahl[1] was until recent years the only method available for the quantitative determination of the total serum protein content or the fractions of albumin, globulin, fibrinogen, etc., separated by the use of 21.5 per cent sodium sulphate at 37° C.[2] or saturated solution at 33° C.[3] or ammonium sulphate.[4] Clinical investigations were consequently few and far from complete. The Kjeldahl still remains the standard method and the court of last appeal, but the introduction of a much simpler, albeit less accurate, colorimetric method by Greenberg[5] stimulated clinical interest and investigation of the serum protein fractions. Exton[6] has developed a scopometer with photoelectric cells for the rapid estimation of the blood proteins according to the turbidity produced, and most recently Page and Van Slyke[7] have advocated a rapid way of determining whether the proteins are below the edema level.

Christian[8] in the Billing's lecture of a year ago discussed the clinical significance of the blood proteins, especially their osmotic pressure value versus the intracapillary pressure in the production of edema, stressing the relation of oligoproteinemia to the accumulation of edema in renal disease.

The importance of the loss of quantities of serum albumin and globulin through the kidneys has long been recognized, and Epstein's[9] high protein diet, in order to make up for this loss and to maintain the blood protein levels and overcome obstinate edema, was the first step in the rational management of such cases and the first application of Starling's[10] conceptions of the function of blood serum proteins.

The so-called "nephrosis syndrome" is the striking example of the effect of a depleted blood serum as a result of a high grade albuminuria. The albumin fraction is of smaller molecular size and is chiefly lost; there is apparently an attempt at compensation or an increase in the globulin fraction, but this Govaerts[11] has shown to be of much less of an osmotic pressure or oncotic value and consequently the hydrophilic power of the blood serum is decreased.

Govaerts[11] has estimated that a one gram per cent solution of albumin in serum exerts an osmotic pressure of 5.5 mm. Hg (74.8 mm. H_2O) while that of a one gram per cent of globulin in serum is only 1.4 mm. Hg (19 mm. H_2O). Normal human serum contains about 4.5 gm. of albumin and 2.5 gm. of globulin, a total serum protein of 7 gm. which exerts an oncotic pressure of about 28 mm. Hg. (380 mm. H_2O). Krogh,[12] Starling had suggested the existence of an equilibrium of this protein osmotic pressure and the intravascular pressure as early as 1896.

The blood proteins are significant in other conditions and may be lost as Turner[13] has pointed out, through other channels, as through the damaged atrophic gastrointestinal tract in pellagra. In pellagra without or with edema, however, as it may also be in wet beri-beri and in waterlogged adult scurvy (a case of which we recently found to have oligoproteinemia) and, as reported by Youmans,[14] in chronic undernutritional states due to diets insufficient in proteins and especially protein of good biologic value, the oligoproteinemia may be the result of an inadequate protein ingestion or assimilation.

Absorption of digested protein may be interfered with by the chronic passive congestion of the mucosa of the gastrointestinal canal, but in congestive heart failure there may be as was suggested[15] another factor responsible, namely, an interference with the elaboration of blood proteins in the engorged liver. This is, of course, merely a surmise, depending upon the fact that it seems established that the fibrinogen fraction is formed in the liver. In congestive heart failure,[15] the edema is due primarily to stasis, and oligoproteinemia plays only a minor but sometimes a significant rôle in the exaggeration and persistence.

Significant diagnostic and prognostic serum albumin-globulin relations in liver disease with ascites and edema have been reported by Signorelli,[16] but the most striking clinical oligoproteinemias are encountered in the nephrotic syndrome. Leiter Alexis Hartmann and his coworkers[17] have recently reported favorable diuresis from injections of acacia in chronic resistantly edematous nephrotic children with oligoproteinemia. They were able to raise the oncotic pressure to normal with the colloid solution. This interesting and important field of the blood serum proteins has been made accessible for clinical investigation by modern methods.[18, 1, 5, 6, 7]

REFERENCES

1. Peters, J. P., and Van Slyke, D. D.: The Kjeldahl Method—Methods—Quantitative Clinical Chemistry, Vol. II, Chapter X, page 516.
2. Howe, P. E.: J. Biol. Chem. **49**: 93, 1921.
3. Oudenal: Dissertation Leiden, 1928.
4. Lewith and Hoffmeister: Arch. Exper. Path. & Pharmacol. **24**: 1 and 247.
5. Greenberg, D. M.: The Colorimetric Determination of the Serum Proteins, J. Biol. Chem. **82**: 545, 1929.
6. Exton, W. G., and Rose, A. R.: Partition of Blood Protein by Scopometry, J. A. M. A. **99**: 1236, 1932.
7. Page, I. H., and Van Slyke, D. D.: A Simple Test for Plasma Protein, J. A. M. A. **99**: 1344, 1932.
8. Christian, H. A.: The Mechanism of Edema in Relation to the Clinical Classification of Bright's Disease (Nephritis), J. A. M. A. **97**: 296, 1931.
9. Epstein, A. A.: Further Observation on the Nature and the Treatment of Chronic Nephrosis, Am. J. M. Sc. **163**: 167, 1922.
10. Starling, L. A.: The Absorption of Fluids From Connective Tissue Spaces, J. Physiol. **19**: 312, 1895-1896.
11. Govaerts, P.: Compt. rend. Soc. de Biol. **93**: 441, 1925.
12. Krogh, A.: The Anatomy and the Physiology of the Capillaries (New Haven), The Yale University Press, 1929, p. 286.
13. Turner, R. H.: The Pathological Physiology of Pellagra, J. Clin. Invest. **10**: 71, 1931.
14. Youmans, J. B.: Endemic Nutritional Edema, J. A. M. A. **88**: 883, 1932.
15. Herrmann, George: Plasma Proteins, Serum Calcium, and Inorganic Phosphorus in Cardiac Edema and After Diuresis, Proc. Soc. Exper. Biol. & Med. **28**: 263, 1930; Observations on the Relation of Some Blood Chemical Findings to Cardiac Function, Plasma Proteins, in Congestive Heart Failure, Trans. Assn. Am. Phys. **46**: 360, 1931; Potassium, Calcium, Inorganic Phosphates and Serum Proteins in Congestive Heart Failure Before and After Treatment, Southern M. J. **25**: 934, 1932.
16. Signorelli, S.: Il Rapporte Siero-albumine, Rio. Clin. Med. **32**: 17, 1931.
17. Hartmann, Alexis, Senn, M. J. L., Nelson, M. V., and Perley, M. A.: The Use of Acacia in the Treatment of Edema, J. A. M. A. **100**: 251, 1932.
18. Bendien, S. G. T., and Piney, L. A.: Specific Changes in Blood Serum, St. Louis, 1932, The C. V. Mosby Co.

—G. H.